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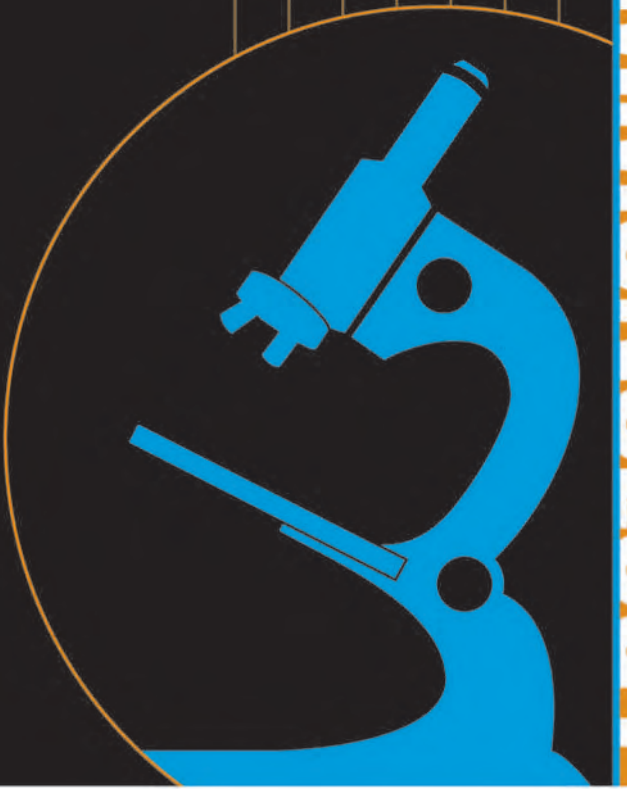
MICROBIOLOGY AND IMMUNOLOGY



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WORLD *of*

MICROBIOLOGY AND IMMUNOLOGY

WORLD *of* MICROBIOLOGY AND IMMUNOLOGY

Brigham Narins, *Editor*

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World of Microbiology and Immunology

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Contents

Introduction vii

How to Use This Book ix

Acknowledgments xiii

Entries

Volume 1: A-L 1

Volume 2: M-Z 359

Sources Consulted 619

Historical Chronology 643

General Index 661

INTRODUCTION

Although microbiology and immunology are fundamentally separate areas of biology and medicine, they combine to provide a powerful understanding of human health and disease—especially with regard to infectious disease, disease prevention, and tragically, of the growing awareness that bioterrorism is a real and present worldwide danger.

World of Microbiology and Immunology is a collection of 600 entries on topics covering a range of interests—from biographies of the pioneers of microbiology and immunology to explanations of the fundamental scientific concepts and latest research developments. In many universities, students in the biological sciences are not exposed to microbiology or immunology courses until the later half of their undergraduate studies. In fact, many medical students do not receive their first formal training in these subjects until medical school. Despite the complexities of terminology and advanced knowledge of biochemistry and genetics needed to fully explore some of the topics in microbiology and immunology, every effort has been made to set forth entries in everyday language and to provide accurate and generous explanations of the most important terms. The editors intend *World of Microbiology and Immunology* for a wide range of readers. Accordingly, the articles are designed to instruct, challenge, and excite less experienced students, while providing a solid foundation and reference for more advanced students. The editors also intend that *World of Microbiology and Immunology* be a valuable resource to the general reader seeking information fundamental to understanding current events.

Throughout history, microorganisms have spread deadly diseases and caused widespread epidemics that threatened and altered human civilization. In the modern era, civic sanitation, water purification, immunization, and antibiotics have dramatically reduced the overall morbidity and the mortality of disease in advanced nations. Yet much of the world is still ravaged by disease and epidemics, and new threats constantly appear to challenge the most advanced medical and public health systems. For all our science and technology, we are far from mastering the microbial world.

During the early part of the twentieth century, the science of microbiology developed somewhat independently of other biological disciplines. Although for many years it did not exist as a separate discipline at all—being an "off-shoot" of chemistry (fermentation science) or medicine—with advances in techniques such as microscopy and pure culturing methodologies, as well as with the establishment of the germ theory of disease and the rudiments of vaccination, microbiology suddenly exploded as a separate discipline. Whereas other biological disciplines were concerned with such topics as cell structure and function, the ecology of plants and animals, the reproduction and development of organisms, the nature of heredity and the mechanisms of evolution, microbiology had a very different focus. It was concerned primarily with the agents of infectious disease, the immune response, the search for chemotherapeutic agents and bacterial metabolism. Thus, from the very beginning, microbiology as a science had social applications. A more detailed historical perspective of the development of the field may be found in the article "History of Microbiology" in this volume.

Microbiology established a closer relationship with other biological disciplines in the 1940s because of its association with genetics and biochemistry. This association also laid the foundations for the subsequent and still rapidly developing field of genetic engineering, which holds promise of profound impact on science and medicine.

Microorganisms are extremely useful experimental subjects because they are relatively simple, grow rapidly, and can be cultured in large quantities. George W. Beadle and Edward L. Tatum studied the relationship between genes and enzymes in 1941 using mutants of the bread mold *Neurospora*. In 1943 Salvador Luria and Max Delbrück used bacterial mutants to show that gene mutations were apparently spontaneous and not directed by the environment. Subsequently, Oswald Avery, Colin M. MacLeod, and Maclyn McCarty provided strong evidence that DNA was the genetic material and carried genetic information during transformation. The interactions between microbiology, genetics, and biochemistry soon

led to the development of modern, molecularly oriented genetics.

Recently microbiology has been a major contributor to the rise of molecular biology, the branch of biology dealing with the physical and chemical bases of living matter and its function. Microbiologists have been deeply involved in studies of the genetic code and the mechanisms of DNA, RNA, and protein synthesis. Microorganisms were used in many of the early studies on the regulation of gene expression and the control of enzyme activity. In the 1970s new discoveries in microbiology led to the development of recombinant gene technology and genetic engineering. One indication of the importance of microbiology today is the number of Nobel Prizes awarded for work in physiology and medicine during the twentieth century; about a third of these were awarded to scientists working on microbiological problems.

Microorganisms are exceptionally diverse, are found almost everywhere, and affect human society in countless ways. The modern study of microbiology is very different from the chemically and medically oriented discipline pioneered by Louis Pasteur and Robert Koch. Today it is a large discipline with many specialities. It has impact on medicine, agricultural and food sciences, ecology, genetics, biochemistry, and many other fields. Today it clearly has both basic and applied aspects.

Many microbiologists are interested in the biology of the microorganisms themselves. They may focus on a specific group of microorganisms and be called virologists (scientists who study viruses), bacteriologists (scientists who study bacteria), phycologists or algologists (scientists who study algae), mycologists (scientists who study fungi), or protozoologists (scientists who study protozoa). Others may be interested in microbial morphology or particular functional processes and work in fields such as microbial cytology, physiology, ecology, genetics, taxonomy, and molecular biology. Some microbiologists may have a more applied orientation and work on problems in fields such as medical microbiology, food and dairy microbiology, or public health. Because the various fields of microbiology are interrelated, an applied microbiologist must always be familiar with basic microbiology. For example, a medical microbiologist must have a good understanding of microbial taxonomy, genetics, immunology, and physiology to identify and properly respond to the pathogen of concern.

It is clear that scientists study the microbial world in much the same way as they studied the world of multicellular organisms at the beginning of the twentieth century, when microbiology was a young discipline. This is in part due to the huge developments and refinements of techniques, which now allow scientists to more closely and fully investigate the world of bacteria and viruses.

One of the focuses of this book is the field of medical microbiology and its connection with immunology. Medical microbiology developed between the years 1875 and 1918, during which time many disease-causing bacteria were identified and the early work on viruses begun. Once people realized that these invisible agents could cause disease, efforts were made to prevent their spread from sick to healthy people. The great successes that have taken place in the area of human health in the past 100 years have resulted largely from advances in the prevention and treatment of infectious disease. We can consider the eradication of smallpox, a viral disease, as a prime example. The agent that causes this disease is one of the greatest killers the world has ever known—and was probably the greatest single incentive towards the formalization of the specialized study of immunology. Research into the mechanism of Edward Jenner's "vaccination" discovery—he found that of a patient injected with cow-pox produces immunity to smallpox—laid the foundations for the understanding of the immune system and the possibility of dealing with other diseases in a similar way. Because of an active worldwide vaccination program, no cases of smallpox have been reported since 1977. (This does not mean, however, that the disease cannot reappear, whether by natural processes or bioterror.)

Another disease that had a huge social impact was bubonic plague, a bacterial disease. Its effects were devastating in the Middle Ages. Between 1346 and 1350, one third of the entire population of Europe died of bubonic plague. Now generally less than 100 people die each year from this disease. The discovery of antibiotics in the early twentieth century provided an increasingly important weapon against bacterial diseases, and they have been instrumental in preventing similar plague epidemics.

Although progress in the application of immunological research has been impressive, a great deal still remains to be done, especially in the treatment of viral diseases (which do not respond to antibiotics) and of the diseases prevalent in developing countries. Also, seemingly "new" diseases continue to arise. Indeed, there has been much media coverage in the past twenty years in the U.S. of several "new" diseases, including Legionnaires' disease, toxic shock syndrome, Lyme disease, and acquired immunodeficiency syndrome (AIDS). Three other diseases emerged in 1993. In the summer of that year a mysterious flu-like disease struck the Southwest, resulting in 33 deaths. The causative agent was identified as a virus, hantavirus, carried by deer mice and spread in their droppings. In the same year, more than 500 residents of the state of Washington became ill with a strain of *Escherichia coli* present in undercooked beef prepared at a fast-food restaurant. The organism synthesized a potent toxin and caused haemolytic-uremic syndrome. Three children died. In 1993, 400,000 people in Milwaukee became ill with a diarrheal disease, cryptosporidiosis, that resulted from the improper chlorination of the water supply.

It is a great credit to the biomedical research community that the causative agents for all these diseases were identified very soon after the outbreaks. The bacteria causing Legionnaires' disease and Lyme disease have only been isolated in the past few decades, as have the viruses that cause AIDS. A number of factors account for the fact that seemingly "new" diseases arise almost spontaneously, even in industrially advanced countries. As people live longer, their ability to ward off infectious agents is impaired and, as a result, the organisms that usually are unable to cause disease become

potentially deadly agents. Also, lifestyles change and new opportunities arise for deadly agents. For example, the use of vaginal tampons by women has resulted in an environment in which the Staphylococcus bacterium can grow and produce a toxin causing toxic shock syndrome. New diseases can also emerge because some agents have the ability to change abruptly and thereby gain the opportunity to infect new hosts. It is possible that one of the agents that causes AIDS arose from a virus that at one time could only infect other animals.

Not only are new diseases appearing but many infectious diseases that were on the wane in the U.S. have started to increase again. One reason for this resurgence is that thousands of U.S. citizens and foreign visitors enter the country daily. About one in five visitors now come from a country where diseases such as malaria, cholera, plague, and yellow fever still exist. In developed countries these diseases have been largely eliminated through sanitation, vaccination, and quarantine. Ironically, another reason why certain diseases are on the rise is the very success of past vaccination programs: because many childhood diseases (including measles, mumps, whooping cough, and diphtheria) have been effectively controlled in both developed and developing countries, some parents now opt not to vaccinate their children. Thus if the disease suddenly appears, many more children are susceptible.

A third reason for the rise of infectious diseases is that the increasing use of medications that prolong the life of the elderly, and of treatments that lower the disease resistance of patients, generally weaken the ability of the immune system to fight diseases. People infected with human immunodeficiency virus (HIV), the virus responsible for AIDS, are a high-risk group for infections that their immune systems would normally resist. For this reason, tuberculosis (TB) has increased in the U.S. and worldwide. Nearly half the world's population is infected with the bacterium causing TB, though for most people the infection is inactive. However, many thousands of new cases of TB are reported in the U.S. alone, primarily among the elderly, minority groups, and people infected with HIV. Furthermore, the organism causing these new cases of TB is resistant to the antibiotics that were once effective in treating the disease. This phenomenon is the result of the uncontrolled overuse of antibiotics over the last 70 years.

Until a few years ago, it seemed possible that the terrible loss of life associated with the plagues of the Middle Ages or with the pandemic influenza outbreak of 1918 and 1919 would never recur. However, the emergence of AIDS dramatizes the fact that microorganisms can still cause serious, incurable, life-threatening diseases. With respect to disease control, there is still much microbiological research to be done, especially in relation to the fields of immunology and chemotherapy.

Recent advances in laboratory equipment and techniques have allowed rapid progress in the articulation and understanding of the human immune system and of the elegance of the immune response. In addition, rapidly developing knowledge of the human genome offers hope for treatments designed to effectively fight disease and debilitation both by directly attacking the causative pathogens, and by strengthening the body's own immune response.

Because information in immunology often moves rapidly from the laboratory to the clinical setting, it is increasingly important that scientifically literate citizens—those able to participate in making critical decisions regarding their own health care—hold a fundamental understanding of the essential concepts in both microbiology and immunology.

Alas, as if the challenges of nature were not sufficient, the evolution of political realities in the last half of the twentieth century clearly points toward the probability that, within the first half of the twenty-first century, biological weapons will surpass nuclear and chemical weapons as a threat to civilization. Accordingly, informed public policy debates on issues of biological warfare and bioterrorism can only take place when there is a fundamental understanding of the science underpinning competing arguments.

The editors hope that *World of Microbiology and Immunology* inspires a new generation of scientists who will join in the exciting worlds of microbiological and immunological research. It is also our modest wish that this book provide valuable information to students and readers regarding topics that play an increasingly prominent role in our civic debates, and an increasingly urgent part of our everyday lives.

K. Lee Lerner & Brenda Wilmoth Lerner, editors
St. Remy, France
June 2002

Editor's note: *World of Microbiology and Immunology* is not intended to be a guide to personal medical treatment or emergency procedures. Readers desiring information related to personal issues should always consult with their physician. The editors respectfully suggest and recommend that readers desiring current information related to emergency protocols—especially with regard to issues and incidents related to bioterrorism—consult the United States Centers for Disease Control and Prevention (CDC) website at http://www.cdc.gov/.

How to Use the Book

The articles in the book are meant to be understandable by anyone with a curiosity about topics in microbiology or immunology. Cross-references to related articles, definitions, and biographies in this collection are indicated by **bold-faced type**, and these cross-references will help explain and expand the individual entries. Although far from containing a comprehensive collection of topics related to genetics, *World of Microbiology and Immunology* carries specifically selected topical entries that directly impact topics in microbiology and immunology. For those readers interested in genetics, the editors recommend Gale's *World of Genetics* as an accompanying reference. For those readers interested in additional information regarding the human immune system, the editors recommend Gale's *World of Anatomy and Physiology*.

This first edition of *World of Microbiology and Immunology* has been designed with ready reference in mind:

- **Entries are arranged alphabetically** rather than chronologically or by scientific field. In addition to classical topics, *World of Microbiology and Immunology* contains many articles addressing the impact of

advances in microbiology and immunology on history, ethics, and society.
- **Bold-faced terms** direct the reader to related entries.
- **"See also" references** at the end of entries alert the reader to related entries not specifically mentioned in the body of the text.
- A **Sources Consulted** section lists the most worthwhile print material and web sites we encountered in the compilation of this volume. It is there for the inspired reader who wants more information on the people and discoveries covered in this volume.
- The **Historical Chronology** includes many of the significant events in the advancement of microbiology and immunology. The most current entries date from just days before *World of Microbiology and Immunology* went to press.
- A **comprehensive General Index** guides the reader to topics and persons mentioned in the book. Bolded page references refer the reader to the term's full entry.

Although there is an important and fundamental link between the composition and shape of biological molecules and their functions in biological systems, a detailed understanding of biochemistry is neither assumed or required for *World of Microbiology and Immunology.* Accordingly, students and other readers should not be intimidated or deterred by the complex names of biochemical molecules (especially the names for particular proteins, enzymes, etc.). Where necessary, sufficient information regarding chemical structure is provided. If desired, more information can easily be obtained from any basic chemistry or biochemistry reference.

Advisory Board

In compiling this edition we have been fortunate in being able to rely upon the expertise and contributions of the following scholars who served as academic and contributing advisors for *World of Microbiology and Immunology*, and to them we would like to express our sincere appreciation for their efforts to ensure that *World of Microbiology and Immunology* contains the most accurate and timely information possible:

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Robert G. Best, Ph.D.
Immunodeficiency disease syndromes
Immunodeficiency diseases, genetic

Antonio Farina, M.D., Ph.D.
Reproductive immunology

Brian D. Hoyle, Ph.D.
Anthrax, terrorist use of as a biological weapon

Eric v.d. Luft, Ph.D., M.L.S.
The biography of Dr. Harry Alfred Feldman

Danila Morano, M.D.
Rh and Rh incompatibility

Judyth Sassoon, Ph.D.
BSE and CJD disease, ethical issues and socio-economic impact

Constance K. Stein, Ph.D.
Genetic identification of microorganisms

Susan Thorpe-Vargas, Ph.D
Immunology, nutritional aspects

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A

ABBE, ERNST (1840-1905)

German optical engineer

Ernst Abbe was among the first optical engineers, designing and perfecting methods for manufacturing microscopes and lens systems of high quality. Though he was a great scientist in his own right, he might have remained anonymous but for the foresight of his employer, Carl Zeiss (1816–1888). In his early twenties Abbe was working as a lecturer in Jena, Germany. He was recognized as being intelligent and industrious, particularly in mathematics, but he was unable to secure a professorial position at the university. In 1855 Zeiss, the owner and operator of a local company that built optical instruments, approached him. Zeiss had realized that the dramatic rise in scientific interest and research in Europe would create a demand for precision instruments—instruments his shop could easily provide. However, neither Zeiss nor his employees possessed the scientific knowledge to design such instruments. Abbe was hired as a consultant to mathematically design lenses of unrivaled excellence.

The science of lenscrafting had stalled since the time of **Anton van Leeuwenhoek** (1632–1723), chiefly due to certain seemingly insurmountable flaws in man-made lenses. Foremost among these was the problem of chromatic aberration, which manifested itself as colored circles around the subject. Scientists were also frustrated with the poor quality of the glass used to make lenses. During the following decade, Abbe worked on new grinding procedures that might correct chromatic aberration; by combining his efforts with Zeiss's glassmaker, Otto Schott, he eventually succeeded in producing near-flawless scientific lenses of exceptionally high power. These same ten years were profitable ones for Abbe. With the increasing success of the Zeiss Works, Abbe was recognized as a scientist and was given a professorship at Jena University in 1875. Zeiss, who realized that the success of his business was in no small part due to Abbe's efforts, made the young professor a partner in 1876. Abbe's work on theoretical optics earned him international notoriety, and he was offered a position at the prestigious University of Berlin (a position he declined in order to continue his research at Zeiss).

During their collaboration Abbe and Zeiss produced thousands of scientific optical instruments. Their innovations set important standards for the development of telescopes and photographic equipment. Carl Zeiss died in 1888 leaving the entire Zeiss Works to Abbe. In addition to running the company, Abbe used his own considerable funds to set up the Carl Zeiss Foundation, an organization for the advancement of science and social improvement.

See also History of microbiology; Microscope and microscopy

ACNE, MICROBIAL BASIS OF

Acne is a condition that affects the hair follicles. A hair follicle consists of a pore the opens to the surface of the skin. The pore leads inward to a cavity that is connected to oil glands. The glands, which are called sebaceous glands, produce oil (sebum) that lubricates the skin and the hair that grows out of the cavity. As the hair grows the oil leaves the cavity and spreads out over the surface of the skin, were it forms a protective coating. However, in conditions such as acne, the oil becomes trapped in the cavities of the hair follicles. This accumulation of oil is irritating and so causes an **inflammation**. One consequence of the inflammation is an unsightly, scabby appearing crust on the surface of the skin over the inflamed follicles. This surface condition is acne.

Acne is associated with the maturation of young adults, particularly boys. Part of the maturation process involves the production or altered expression of hormones. In adolescence certain hormones called androgens are produced. Androgens stimulate the enlargement of the sebaceous glands and the resulting production of more oil, to facilitate the manufacture of more facial hair. In girls, androgen production is greater around the time of menstruation. Acne often appears in young women at the time of their monthly menstrual period.

Facial acne caused by *Propionibacterium acne*.

In this altered hormonal environment, **bacteria** play a role in the development of acne. The principal bacterial species associated with acne is *Proprionibacterium acnes*. This microorganism is a normal resident on the skin and inside hair follicles. Normally, the outward flow of oil will wash the bacteria to the surface and be removed when the face is washed. However, in the androgen-altered hair follicles, the cells lining the cavity shed more frequently, stick together, mix with the excess oil that is being produced, and pile up in clumps inside the cavity. The accumulated material is a ready nutrient source for the *Proprionibacterium acnes* in the cavity. The bacteria grow and multiply rapidly.

Two other bacterial species that live and grow on the surface of the skin can be associated with acne. These are *Proprionibacterium granulosum* and *Staphylocccus epidermidis*. Their significance is less than *Proprionibacterium acnes*, however.

As the numbers of bacteria increase, the by-products of their metabolic activities cause even more inflammation. Also, the bacteria contain **enzymes** that can degrade the oil from the oil glands into what are known as free fatty acids. These free fatty acids are very irritating to the skin. Various other bacterial enzymes contribute to inflammation, including proteases and phosphatases.

The **immune system** does react to the abnormal growth of the bacteria by trying to clear the bacteria. Death of bacteria combined with the immune response generates the material known as pus. A hallmark of acne is often the pus that is exuded from the crusty sores on the skin.

The altered environment within the hair follicle that facilitates the explosive growth of *Proprionibacterium acnes* can be stimulated by factors other than the altered hormone production of puberty. The external environment, particularly a warm and moist one, is one factor.

The damage caused by bacteria in acne ranges from mild to severe. In a mild case of acne, only a so-called blackheads or whiteheads are evident on the skin. More severe cases are associated with more blackheads, whiteheads and pimples, and also with inflammation. The most severe form, called cystic acne, may produce marked inflammation over the entire upper body, and requires a physician's attention to reduce the bacterial populations.

Reduction in the bacterial number involves slowing down the secretion of the oil from the oil glands and making the follicle pore more open, so that the normal outward flow can occur. Oil production can be slowed in the presence of 12-cis-retinoic acid (Accutane). Use of this medication is reserved for severe cases of acne, as the retinoic acid can have significant adverse side effects. Antibacterial agents can also be useful. For example, many antibacterial creams and face washes contain the compound called benzoyl peroxide, which is very active against *Proprionibacterium acnes.*

Because the bacteria active in acne are normal residents of the skin, there is no "cure" for acne. Rather, the condition is lessened until biochemical or lifestyle changes in the individual lessen or eliminate the conditions that promote bacterial overgrowth.

See also Microbial flora of the skin; Skin infections

ACRIDINE ORANGE

Acridine orange is a fluorescent dye. The compound binds to genetic material and can differentiate between **deoxyribonucleic acid** (**DNA**) and **ribonucleic acid** (**RNA**).

A fluorescent dye such as acridine orange absorbs the energy of incoming light. The energy of the light passes into the dye molecules. This energy cannot be accommodated by the dye forever, and so is released. The released energy is at a different wavelength than was the incoming light, and so is detected as a different color.

Acridine orange absorbs the incoming radiation because of its ring structure. The excess energy effectively passes around the ring, being distributed between the various bonds that exist within the ring. However, the energy must be dissipated to preserve the stability of the dye structure.

The ring structure also confers a **hydrophobic** (water-hating) nature to the compound. When applied to a sample in solution, the acridine orange will tend to diffuse spontaneously into the membrane surrounding the **microorganisms**. Once in the interior of the cell, acridine orange can form a complex with DNA and with RNA. The chemistries of these complexes affect the wavelength of the emitted radiation. In the case of the acridine orange–DNA complex, the emitted radiation is green. In the case of the complex formed with RNA, the emitted light is orange. The different colors allow DNA to be distinguished from RNA.

Binding of acridine orange to the nucleic acid occurs in living and dead **bacteria** and other microorganisms. Thus, the dye is not a means of distinguishing living from dead microbes. Nor does acridine orange discriminate between one species of microbe versus a different species. However, acridine orange has proved very useful as a means of enumerating the total number of microbes in a sample. Knowledge of the total number of bacteria versus the number of living bacteria

can be very useful in, for example, evaluating the effect of an antibacterial agent on the survival of bacteria.

Acridine orange is utilized in the specialized type of light microscopic technique called fluorescence microscopy. In addition, fluorescence of DNA or RNA can allow cells in a sample to be differentiated using the technique of flow cytometry. This sort of information allows detailed analysis of the DNA replication cycle in microorganisms such as **yeast**.

See also Laboratory techniques in microbiology

ACTINOMYCES

Actinomyces is a genus of **bacteria**. The bacteria that grouped in this genus share several characteristics. The bacteria are rod-like in shape. Under the light **microscope**, *Actinomyces* appear fungus-like. They are thin and joined together to form branching networks. Bacteria of this genus retain the primary stain in the Gram stain reaction, and so are classified as being Gram positive. *Actinomycetes* are not able to form the dormant form known as a spore. Finally, the bacteria are able to grow in the absence of oxygen.

Members of the genus *Actinomyces* are normal residents of the mouth, throat, and intestinal tract. But they are capable of causing infections both in humans and in cattle if they are able to enter other regions. This can occur as the result of an accident such as a cut or abrasion.

An infection known as *Actinomycosis* is characterized by the formation of an abscess—a process "walling off" the site of infection as the body responds to the infection—and by swelling. Pus can also be present. The pus, which is composed of dead bacteria, is granular, because of the presence of granules of sulfur that are made by the bacteria.

The diagnosis of an *Actinomyces* infection can be challenging, as the symptoms and appearance of the infection is reminiscent of a tumor or of a **tuberculosis** lesion. A well-established infection can produce a great deal of tissue damage. Additionally, the slow growth of the bacteria can make the treatment of infection with **antibiotics** very difficult, because antibiotics rely on **bacterial growth** in order to exert their lethal effect.

The culturing of *Actinomyces* in the laboratory is also challenging. The bacteria do not grow on nonselective media, but instead require the use of specialized and nutritionally complex selective media. Furthermore, incubation needs to be in the absence of oxygen. The growth of the bacteria is quite slow. Solid growth medium may need to be incubated for up to 14 days to achieve visible growth. In contrast, a bacterium like ***Escherichia coli*** yields visible colonies after overnight growth on a variety of nonselective media. The colonies of Actinomyces are often described as looking like bread crumbs.

Currently, identification methods such as **polymerase chain reaction** (**PCR**), chromatography to detect unique cell wall constituents, and antibody-based assays do always perform effectively with *Actinomyces*.

See also Anaerobes and anaerobic infections; Microbial flora of the oral cavity, dental caries

ACTIVE TRANSPORT • *see* CELL MEMBRANE TRANSPORT

ADENOVIRUSES

Adenoviruses are **viruses** which have twenty sides. As such they are called icosahedrons. The outer surface, the capsid, is made of particles of a protein. The protein is arranged in groups of six (hexagons) except at the twenty points where the sides meet (each is called an apex), where the particles are in a pentagon arrangement. A so-called penton fibre, which resembles a stick with a ball at the end, protrudes from each apex.

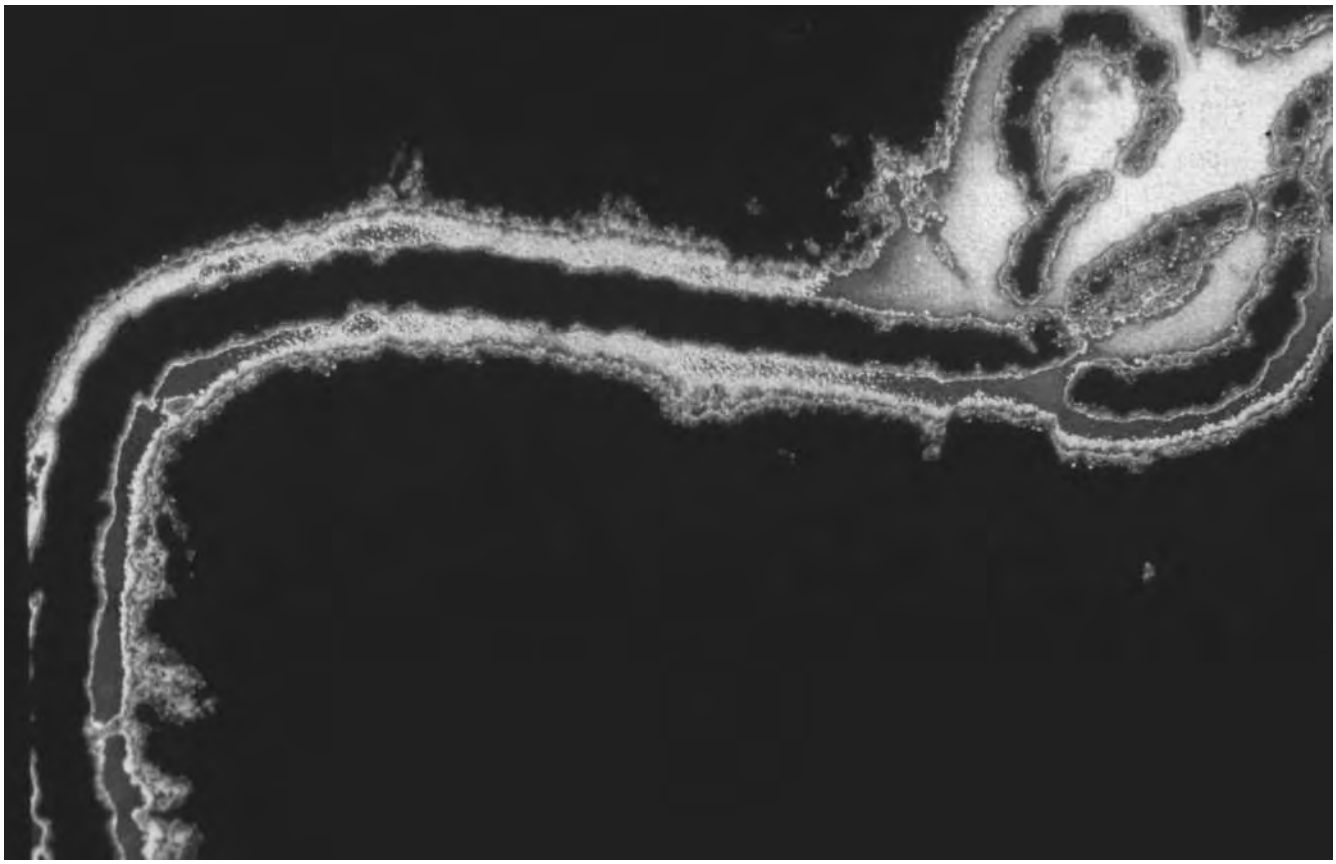
Adenoviruses contain **deoxyribonucleic acid** (DNA) as their genetic material. The **DNA** encodes 20 to 30 proteins, 15 of which are proteins that form the structure of the virus particle. Similar to other viruses, adenoviruses invade a host cell and use the host genetic machinery to manufacture new virus particles. The new viruses are released from the host cell.

Children suffer from adenovirus infections much more so than adults.

The viruses of this group infect the membranes that line the respiratory tract, the eyes, the intestines, and the urinary tract. The adenoviruses that infect humans usually cause mild maladies, including respiratory and intestinal illnesses and conjunctivitis (an **inflammation** of eye membrane, which is also commonly called "pink eye"). A more severe eye malady called keratoconjunctivitis can more widely infect the eye. The **eye infections** are very contagious and are typically a source of transmission of adenovirus from one person to another. Children can also develop a sore throat, runny nose, cough and flu-like illness. Bronchitis, an inflammation of the membranes lining the air passages in the lungs, can also result from adenovirus infection, as can an inflammation of the stomach called **gastroenteritis**. Urinary tract infections can cause pain and burning upon urination and blood in the urine. In dogs, adenovirus type 2 causes what is known as kennel cough. But curiously, the virus also protects dogs against **hepatitis**.

In the setting of the laboratory, some of the human strains of adenovirus can transform cells being grown in cell **culture**. Transformed cells are altered in their regulation of growth, such that the unrestricted growth characteristic of cancers occurs.

Adenoviruses have been known since the mid-1950s. They were first isolated from infected tonsils and adenoidal tissue in 1953. Within the next several years they had been obtained from cells involved in respiratory infections. In 1956, the multiple antigenic forms of the virus that had been discovered were classified as adenovirus. Then, in 1962, laboratory studies demonstrated that an adenovirus caused tumors in



Negative stain electron micrograph of an Adenovirus.

rodents. This was the first known human virus capable of inducing tumors in animals.

More recently, the basis of the tumor-inducing activity has been unraveled. Genes that are active early in the replication cycle of adenovirus produce proteins that interfere with host proteins that are known as anti-oncogenes. Normally, the anti-oncogen proteins are responsive to cell growth, and so act as a signal to the cell to halt growth. By disrupting the anti-oncogene proteins, this stop signal is eliminated, resulting in the continued and uncontrolled growth of the cell. A tumor is produced. Thus, adenoviruses have become important as one of the central triggers of cancer development.

Such cancers may be a by-product of adenovirus infections. These infections are not by themselves serious. Most tend to appear and run their course within a few weeks. The infections are fairly common. For example, most children will have antibodies to at least four types of adenovirus. Adenovirus gains entry through a break in the skin or are inhaled. The stick-and-ball appearing penton fibers may have a role in the attachment of the virus particle to a protein on the surface of the host epithelial cell.

Adenovirus infections have contributed to the spread of bacterial **antibiotic resistance** because of the overuse of **antibiotics**. The flu-like symptoms of some adenovirus infections can lead to the prescribing of antibiotics as a treatment. However, antibiotics are ineffective against viruses. But the circulating antibiotic can provide selective pressure on the development of resistant in bacterial populations.

See also Bacterial adaptation; Transformation

ADJUVANT

An adjuvant is any substance that enhances the response of the **immune system** to the foreign material termed an **antigen**. The particular antigen is also referred to as an immunogen. An adjuvant can also be any substance that enhances the effect of a drug on the body.

When antigen is injected into an organism being used to raise antibodies the effect is to stimulate a greater and more prolonged production of **antibody** than would otherwise occur if the antigen were injected alone. Indeed, adjuvants are very useful if a substance itself is not strongly recognized by the immune system. An example of such a weak immunogen is the capsule exopolysaccharide of a variety of **bacteria**.

Adjuvants exert their effect in several different ways. Firstly, some adjuvants retain the antigen and so "present" the antigen to the immune system over a prolonged period of time. The immune response does not occur all at once, but rather is

continuous over a longer time. Secondly, an adjuvant itself can react with some of the cells of the immune system. This interaction may stimulate the immune cells to heightened activity. Thirdly, an adjuvant can also enhance the recognition and ingestion of the antigen by the immune cell known as the **phagocyte**. This enhanced **phagocytosis** presents more antigens to the other cells that form the antibody.

There are several different types of antigens. The adjuvant selected typically depends on the animal being used to generate the antibodies. Different adjuvants produce different responses in different animals. Some adjuvants are inappropriate for certain animals, due to the **inflammation**, tissue damage, and pain that are caused to the animal. Other factors that influence the choice of an adjuvant include the injection site, the manner of antigen preparation, and amount of antigen injected.

One type of adjuvant that has been of long-standing service in generating antibodies for the study of bacteria is known as Freund's Complete Adjuvant. This type of adjuvant enhances the response to the immunogen of choice via the inclusion of a type of bacteria called mycobacteria into a mixture of oil and water. Typically, there is more oil present than water. The oil and water acts to emulsify, or spread evenly throughout the suspension, the mycobacteria and the immunogen. Sometimes the mycobacteria are left out of the adjuvant. In this case, it is referred to as "incomplete" adjuvant.

See also Immunity: active, passive, and delayed

AEROBES

Aerobic **microorganisms** require the presence of oxygen for growth. Molecular oxygen functions in the respiratory pathway of the microbes to produce the energy necessary for life. **Bacteria**, yeasts, **fungi**, and algae are capable of aerobic growth.

The opposite of an aerobe is an anaerobe. An anaerobe does not require oxygen, or sometimes cannot even tolerate the presence of oxygen.

There are various degrees of oxygen tolerance among aerobic microorganisms. Those that absolutely require oxygen are known as obligate aerobes. Facultative aerobes prefer the presence of oxygen but can adjust their metabolic machinery so as to grow in the absence of oxygen. Microaerophilic organisms are capable of oxygen-dependent growth but cannot grow if the oxygen concentration is that of an air atmosphere (about 21% oxygen). The oxygen content must be lower.

Oxygen functions to accept an electron from a substance that yields an electron, typically a substance that contains carbon. Compounds called flavoproteins and cytochromes are key to this electron transport process. They act as electron carriers. By accepting an electron, oxygen enables a process known as catabolism to occur. Catabolism is the breakdown of complex structures to yield energy. The energy is used to sustain the microorganism.

A common food source for microorganisms is the sugar glucose. Compounds such as glucose store energy inside themselves, in order to bond their constituent molecules together. When these bonds are severed, energy is released. In aerobic bacteria and other organisms, a compound called pyruvic acid retains most of the energy that is present in the glucose. The pyruvic acid in turn is broken down via a series of reactions that collectively are called the tricarboxylic acid cycle, or the Kreb's cycle (named after one the cycle's discoverers, Sir Hans Krebs). A principle product of the Kreb's cycle is a compound called nicotinamide adenine dinucleotide ($NADH_2$). The $NADH_2$ molecules feed into another chain of reactions of which oxygen is a key.

The energy-generating process in which oxygen functions is termed aerobic **respiration**. Oxygen is the final electron acceptor in the process. Anaerobic respiration exists, and involves the use of an electron acceptor other than oxygen. One of the most common of these alternate acceptors is nitrate, and the process involving it is known as denitrification.

Aerobic respiration allows a substrate to be broken down (this is also known as oxidation) to carbon dioxide and water. The complete breakdown process yields 38 molecules of adenine triphosphate (ATP) for each molecule of the sugar glucose. ATP is essentially the gasoline of the cell. Electron transport that does not involve oxygen also generates ATP, but not in the same quantity as with aerobic respiration. Thus, a facultative aerobe will preferentially use oxygen as the electron acceptor. The other so-called fermentative type of energy generation is a fall-back mechanism to permit the organism's survival in an oxygen-depleted environment.

The aerobic mode of energy production can occur in the disperse **cytoplasm** of bacteria and in the compartmentalized regions of **yeast**, fungi and algae cells. In the latter microorganisms, the structure in which the reactions take place is called the mitochondrion. The activities of the mitochondrion are coordinated with other energy-requiring processes in the cell.

See also Carbon cycle in microorganisms; Metabolism

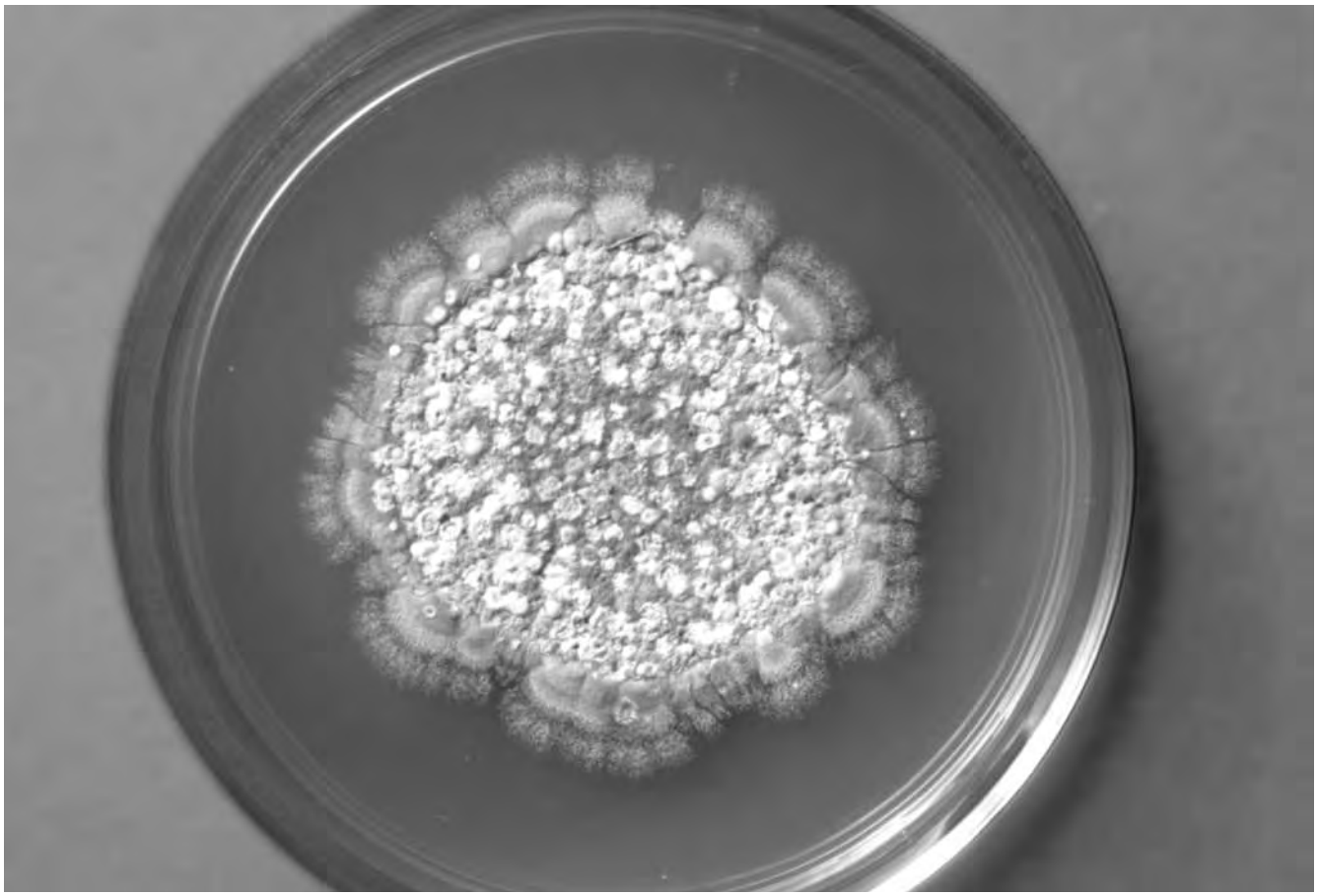
AGAMMAGLOBULINAEMIA WITH HYPER IGM

• *see* IMMUNODEFICIENCY DISEASE SYNDROMES

AGAR AND AGAROSE

Agar and agarose are two forms of solid growth media that are used for the **culture** of **microorganisms**, particularly **bacteria**. Both agar and agarose act to solidify the nutrients that would otherwise remain in solution. Both agar and agarose are able to liquefy when heated sufficiently, and both return to a gel state upon cooling.

Solid media is prepared by heating up the agar and nutrient components so that a solution results. The solution is then sterilized, typically in steam-heat apparatus known as an autoclave. The sterile medium is then poured into one half of sterile Petri plates and the lid is placed over the still hot solution. As the solution cools, the agar or agarose becomes gel-like, rendering the medium in a semi-solid. When bacteria



Aerobic fungus growing on agar.

contact the surface of the medium, they are able to extract the nutrients from the medium and grow as colonies.

The use of agar and agarose solid media allows for the isolation of bacteria by a streak plate technique. A similar discrimination of one bacterial species from another is not possible in liquid growth media. Furthermore, some solid growth media allows reactions to develop that cannot develop in liquid media. The best-known example is **blood agar**, where the total and partial destruction of the constituent red blood cells can be detected by their characteristic hemolytic reactions.

Agar is an uncharged network of strands of a compound called gelactose. This compound is in fact made up of two polysaccharides called agarose and agaropectin. Gelactose is extracted from a type of seaweed known as *Gelidium comeum*. The seaweed was named for the French botanist who first noted the gelatinous material that could be extracted from the **kelp**. Another seaweed called *Gracilaria verrucosa* can also be a source of agar.

Agarose is obtained by purification of the agar. The agarose component of agar is composed of repeating molecules of galactopyranose. The side groups that protrude from the galactopyranose are arranged such that two adjacent chains can associate to form a helix. The chains wrap together so tightly that water can be trapped inside the helix. As more and more helices are formed and become cross-linked, a three-dimensional network of water-containing helices is created. The entire structure has no net charge.

The history of agar and agarose extends back centuries and the utility of the compounds closely follow the emergence and development of the discipline of microbiology. The gel-like properties of agar are purported to have been first observed by a Chinese Emperor in the mid-sixteenth century. Soon thereafter, a flourishing agar manufacturing industry was established in Japan. The Japanese dominance of the trade in agar only ended with World War II. Following World War II, the manufacture of agar spread to other countries around the globe. For example, in the United States, the copious seaweed beds found along the Southern California coast has made the San Diego area a hotbed of agar manufacture. Today, the manufacture and sale of agar is lucrative and has spawned a competitive industry.

The roots of agar as an adjunct to microbiological studies dates back to the late nineteenth century. In 1882, the renowned microbiologist **Robert Koch** reported on the use of agar as a means for growing microorganisms. Since this discovery, the use of agar has become one of the bedrock techniques in microbiology. There are now hundreds of different formulations of agar-based growth media. Some are nonspe-

cific, with a spectrum of components present. Other media are defined, with precise amounts of a few set materials included. Likewise the use of agarose has proved tremendously useful in electrophoretic techniques. By manipulation of the formulation conditions, the agarose matrix can have pores, or tunnels through the agarose strands, which can be of different size. Thus the agarose can act as a sieve, to separate molecules on the basis of the size. The uncharged nature of agarose allows a current to be passed through it, which can drive the movement of samples such as pieces of **deoxyribonucleic acid** (**DNA**) from one end of an agarose slab to the other. The speed of the molecule movement, is also related to molecular size (largest molecules moving the least).

In the non-microbiological world, agar and agarose have also found a use as stabilizers in ice cream, instant cream whips, and dessert gelatins.

See also Bacterial growth and division; Laboratory techniques in microbiology

AGAR DIFFUSION

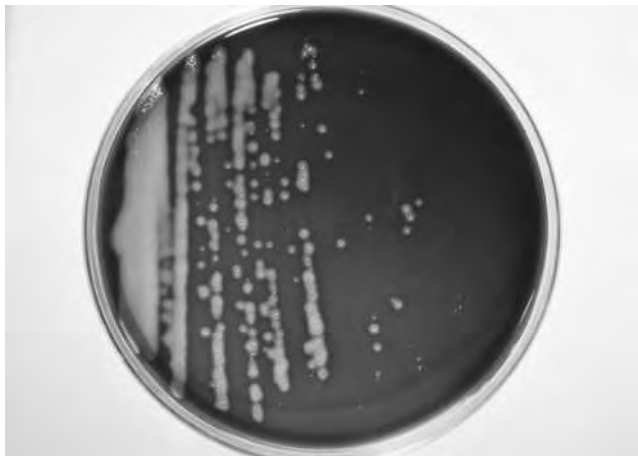
Agar diffusion refers to the movement of molecules through the matrix that is formed by the gelling of agar. When performed under controlled conditions, the degree of the molecule's movement can be related to the concentration of the molecule. This phenomenon forms the basis of the agar diffusion assay that is used to determine the susceptibility or resistance of a bacterial strain to an antibacterial agent, (e.g., including **antibiotics**.

When the seaweed extract known as agar is allowed to harden, the resulting material is not impermeable. Rather, there are spaces present between the myriad of strands of agar that comprise the hardened polymer. Small molecules such as antibiotics are able to diffuse through the agar.

Typically, an antibiotic is applied to a well that is cut into the agar. Thus, the antibiotic will tend to move from this region of high concentration to the surrounding regions of lower antibiotic concentration. If more material is present in the well, then the zone of diffusion can be larger.

This diffusion was the basis of the agar diffusion assay devised in 1944. A bacterial suspension is spread onto the surface of the agar. Then, antibiotic is applied to a number of wells in the plate. There can be different concentrations of a single antibiotic or a number of different antibiotics present. Following a time to allow for growth of the **bacteria** then agar is examined. If **bacterial growth** is right up to the antibiotic containing well, then the bacterial strain is deemed to be resistant to the antibiotic. If there is a clearing around the antibiotic well, then the bacteria have been adversely affected by the antibiotic. The size of the inhibition zone can be measured and related to standards, in order to determine whether the bacterial strain is sensitive to the antibiotic.

This technique can also be done by placing disks of an absorbent material that have been soaked with the antibiotic of interest directly onto the agar surface. The antibiotic will subsequently diffuse out of the disk into the agar. This version of agar diffusion is known as the Kirby-Bauer disk-diffusion assay.



Staphylococcus colonies showing hemolytic reaction on blood agar.

The agar diffusion assay allows bacteria to be screened in a routine, economical and easy way for the detection of resistance. More detailed analysis to ascertain the nature of the resistance can then follow.

See also Antibiotic resistance, tests for; Laboratory techniques in microbiology

AGGLUTINATION • *see* ANTIBODY-ANTIGEN, BIOCHEMICAL AND MOLECULAR REACTIONS

AIDS

The advent of AIDS (acquired **immunity** deficiency syndrome) in early 1981 surprised the scientific community, as many researchers at that time viewed the world to be on the brink of eliminating infectious disease. AIDS, an infectious disease syndrome that suppresses the **immune system**, is caused by the **Human Immune Deficiency Virus** (**HIV**), part of a group of **viruses** known as **retroviruses**. The name AIDS was coined in 1982. Victims of AIDS most often die from opportunistic infections that take hold of the body because the immune system is severely impaired.

Following the discovery of AIDS, scientists attempted to identify the virus that causes the disease. In 1983 and 1984 two scientists and their teams reported isolating HIV, the virus that causes AIDS. One was French immunologist **Luc Montagnier** (1932–), working at the Pasteur Institute in Paris, and the other was American immunologist **Robert Gallo** (1937–) at the National Cancer Institute in Bethesda, Maryland. Both identified HIV as the cause of AIDS and showed the pathogen to be a retrovirus, meaning that its genetic material is **RNA** instead of **DNA**. Following the discov-

ery, a dispute ensued over who made the initial discovery, but today Gallo and Montagnier are credited as co-discoverers.

Inside its host cell, the HIV retrovirus uses an enzyme called reverse transcriptase to make a DNA copy of its genetic material. The single strand of DNA then replicates and, in double stranded form, integrates into the chromosome of the host cell where it directs synthesis of more viral RNA. The viral RNA in turn directs the synthesis protein capsids and both are assembled into HIV viruses. A large number of viruses emerge from the host cell before it dies. HIV destroys the immune system by invading lymphocytes and macrophages, replicating within them, killing them, and spreading to others.

Scientists believe that HIV originated in the region of sub-Saharan Africa and subsequently spread to Europe and the United States by way of the Caribbean. Because viruses exist that suppress the immune system in monkeys, scientists hypothesize that these viruses mutated to HIV in the bodies of humans who ate the meat of monkeys, and subsequently caused AIDS. A fifteen-year-old male with skin lesions who died in 1969 is the first documented case of AIDS. Unable to determine the cause of death at the time, doctors froze some of his tissues, and upon recent examination, the tissue was found to be infected with HIV. During the 1960s, doctors often listed leukemia as the cause of death in many AIDS patients. After several decades however, the incidence of AIDS was sufficiently widespread to recognize it as a specific disease. Epidemiologists, scientists who study the incidence, cause, and distribution of diseases, turned their attention to AIDS. American scientist James Curran, working with the **Centers for Disease Control** and Prevention (CDC), sparked an effort to track the occurrence of HIV. First spread in the United States through the homosexual community by male-to-male contact, HIV rapidly expanded through all populations. Presently new HIV infections are increasing more rapidly among heterosexuals, with women accounting for approximately twenty percent of the AIDS cases. The worldwide AIDS epidemic is estimated to have killed more than 6.5 million people, and infected another 29 million. A new infection occurs about every fifteen seconds. HIV is not distributed equally throughout the world; most afflicted people live in developing countries. Africa has the largest number of cases, but the fastest rate of new infections is occurring in Southeast Asia and the Indian subcontinent. In the United States, though the disease was concentrated in large cities, it has spread to towns and rural areas. Once the leading cause of death among people between the ages of 25 and 44 in the Unites States, AIDS is now second to accidents.

HIV is transmitted in bodily fluids. Its main means of transmission from an infected person is through sexual contact, specifically vaginal and anal intercourse, and oral to genital contact. Intravenous drug users who share needles are at high risk of contracting AIDS. An infected mother has a 15 to 25% chance of passing HIV to her unborn child before and during birth, and an increased risk of transmitting HIV through breast-feeding. Although rare in countries such as the United States where blood is screened for HIV, the virus can be transmitted by transfusions of infected blood or blood-clotting factors. Another consideration regarding HIV transmission is that a person who has had another sexually transmitted disease is more likely to contract AIDS.

Laboratories use a test for HIV-1 that is called **Enzyme-linked immunosorbant assay (ELISA)**. (There is another type of HIV called HIV-2.) First developed in 1985 by Robert Gallo and his research team, the ELISA test is based on the fact that, even though the disease attacks the immune system, **B cells** begin to produce antibodies to fight the invasion within weeks or months of the infection. The test detects the presence of HIV-1 type antibodies and reacts with a color change. Weaknesses of the test include its inability to detect 1) patients who are infectious but have not yet produced HIV-1 antibodies, and 2) those who are infected with HIV-2. In addition, ELISA may give a false positive result to persons suffering from a disease other than AIDS. Patients that test positive with ELISA are given a second more specialized test to confirm the presence of AIDS. Developed in 1996, this test detects HIV antigens, proteins produced by the virus, and can therefore identify HIV before the patient's body produces antibodies. In addition, separate tests for HIV-1 and HIV-2 have been developed.

After HIV invades the body, the disease passes through different phases, culminating in AIDS. During the earliest phase the infected individual may experience general flu-like symptoms such as fever and headache within one to three weeks after exposure; then he or she remains relatively healthy while the virus replicates and the immune system produces antibodies. This stage continues for as long as the body's immune response keeps HIV in check. Progression of the disease is monitored by the declining number of particular antibodies called CD4-T lymphocytes. HIV attacks these immune cells by attaching to their CD4 receptor site. The virus also attacks macrophages, the cells that pass the **antigen** to helper T lymphocytes. The progress of HIV can also be determined by the amount of HIV in the patient's blood. After several months to several years, the disease progresses to the next stage in which the CD4-T cell count declines, and non-life-threatening symptoms such as weakness or swollen lymph glands may appear. The CDC has established a definition for the diagnosis of AIDS in which the CD4 T-cell count is below 200 cells per cubic mm of blood, or an opportunistic disease has set in.

Although progress has been made in the treatment of AIDS, a cure has yet to be found. In 1995 scientists developed a potent cocktail of drugs that help stop the progress of HIV. Among other substances, the cocktail combines zidovudine (AZT), didanosine (ddi), and a protease inhibitor. AZT and ddi are nucleosides that are building blocks of DNA. The enzyme, reverse transcriptase, mistakenly incorporates the drugs into the viral chain, thereby stopping DNA synthesis. Used alone, AZT works temporarily until HIV develops immunity to the nucleoside. Proteases are **enzymes** that are needed by HIV to reproduce, and when protease inhibitors are administered, HIV replicates are no longer able to infect cells. In 1995 the Federal Drug Administration approved saquinaviras, the first protease inhibitor to be used in combination with nucleoside drugs such as AZT; this was followed in 1996 by approval for the protease inhibitors ritonavir and indinavir to be used alone or in combination with nucleosides. The combination of drugs

brings about a greater increase of antibodies and a greater decrease of fulminant HIV than either type of drug alone. Although patients improve on a regimen of mixed drugs, they are not cured due to the persistence of inactive virus left in the body. Researchers are looking for ways to flush out the remaining HIV. In the battle against AIDS, researchers are also attempting to develop a **vaccine**. As an adjunct to the classic method of preparing a vaccine from weakened virus, scientists are attempting to create a vaccine from a single virus protein.

In addition to treatment, the battle against AIDS includes preventing transmission of the disease. Infected individuals pass HIV-laden macrophages and T lymphocytes in their bodily fluids to others. Sexual behaviors and drug-related activities are the most common means of transmission. Commonly, the virus gains entry into the bloodstream by way of small abrasions during sexual intercourse or direct injection with an infected needle. In attempting to prevent HIV transmission among the peoples of the world, there has been an unprecedented emphasis on **public health** education and social programs; it is vitally important to increase public understanding of both the nature of AIDS and the behaviors that put individuals at risk of spreading or contracting the disease.

See also AIDS, recent advances in research and treatment; Antibody and antigen; Blood borne infections; Centers for Disease Control (CDC); Epidemics, viral; Human immunodeficiency virus (HIV); Immunodeficiency disease syndromes; Immunodeficiency diseases; Immunological analysis techniques; Infection and resistance; Infection control; Latent viruses and diseases; Sexually transmitted diseases; T cells or T lymphocytes; Viral genetics; Viral vectors in gene therapy; Virology; Virus replication; Viruses and responses to viral infection

AIDS, RECENT ADVANCES IN RESEARCH AND TREATMENT

Acquired Immune Deficiency Syndrome (**AIDS**) has only been known since the early years of the 1980s. Since that time, the number of people infected with the causative virus of the syndrome and of those who die from the various consequences of the infection, has grown considerably.

In the 1980s and 1990s, researchers were able to establish that the principle target for the maladies associated with AIDS is the **immune system**. Since then, much research has been directed towards pinpointing the changes in the human immune system due to infection, seeking ways of reversing these changes, or supplementing the compromised immune system to hold the infection in check.

The particular immune system component that has been implicated in the progression of AIDS is a type of T cell called the CDC4 T cell. This cell, which is activated following recognition of the virus by the immune system, functions in the destruction of the cells that have been infected by the virus. Over time, however, the number of CDC4 cells declines. If the decline decreases the T cell count to below 200 per microliter of blood, the number of infective virus particles goes up steeply and the immune system breaks down. This loss of the ability to fight off foreign organisms leaves the patient open to life-threatening illnesses that normally would be routinely defeated by an unimpaired immune system.

Until 2001, the prevailing view was that the decline in the number of CDC4 cells was due to a blockage of new T cell production by the infecting virus. However, the conclusions from studies published in 2001 now indicate that the production of new **T cells** is not blocked, but rather that there is acceleration in the loss of existing T cells. Even though the result is the same, namely the increased loss of the specialized AIDS-fighting T cells, the nature of the decline is crucial to determine in order to devise the most effective treatment strategy. If the reasons for the accelerated loss of the T cells can be determined, perhaps the loss can be prevented. This would better equip patients to fight the infection.

Since 1998, a multi-pronged strategy of AIDS therapy has been established. Highly Active Anti-Retroviral Therapy (HAART) consists of administering a "cocktail" of drugs targeted to the AIDS virus to a patient, even when the patient shows no symptoms of AIDS. The drug mixture typically contains a so-called nucleoside analog, which blocks genetic replication, and inhibitors of two **enzymes** that are critical enzyme in the making of new virus (protease and reverse transcriptase).

HAART has greatly reduced the loss of life due to AIDS. But, this benefit has come at the expense of side effects that can often be severe. Also, the treatment is expensive. But now, research published toward the end of 2001 indicates that the use of HAART in a "7-day-on, 7-day-off" cycle does not diminish treatment benefits, but does diminish treatment side effects. Costs of treatment has become more reasonable, as well.

Another advancement in AIDS treatment may come from the finding that the inner core of the AIDS virus, which is called the nucleocapsid, is held together by structures known as "zinc fingers." There are drugs that appear to break apart these supports. This stops the virus from functioning. Furthermore, evidence supports the view that the nucleocapsid does not change much over time. Thus, a drug that effectively targeted the nucleocapsid could be an effective drug for a long time. The drawback to this approach at the present time is that other structures in the body utilize zinc fingers. So, an anti-AIDS zinc finger strategy will have to be made very specific.

In the mid 1980s, there was great optimism that a **vaccine** for the AIDS virus would be developed within two years. However, this optimism soon disappeared. In late 2001, however, preliminary clinical trials began on a candidate vaccine. Traditional vaccines rely on the administration of a protein to stimulate the production of an **antibody** that confers protection against the disease-causing organism. The candidate vaccine works by targeting what is called cell-mediated **immunity**. This type of immunity does not prevent infection, but rather clears the virus-infected cells out of the body. Such a vaccine would be intended to prolong and enhance the quality of the lives of AIDS-infected people. Studies in monkeys have been encouraging. However, studies must still rule out the possibil-

ity that **vaccination** would create "carriers," individuals who are not sick but who are capable of spreading the disease.

There are various vaccine treatment strategies. One involves the injection of so-called "naked" **DNA**. The DNA contains genes that code for *gag*, a viral component thought to be critical to the development of AIDS. The DNA can be attached to inert particles that stimulate the response of the immune system. In another strategy, the viral **gene** is bundled into the DNA of another virus that is injected into the patient.

As of 2002, more than two dozen experimental vaccines intended to control, but not cure, AIDS infections are being studied worldwide.

Treatment strategies, vaccine-based or otherwise, will need to address the different isolates of the AIDS virus that are present in various regions of the globe. These different isolates tend to be separated into different geographical regions. Even within a geographical area, an isolate can display variation from place to place. Thus, it has become clear that a universal treatment strategy is unlikely.

See also Human immunodeficiency virus (HIV); Immune stimulation, as a vaccine; Vaccination

ALEXANDER, HATTIE ELIZABETH (1901-1968)

American physician and microbiologist

Hattie Elizabeth Alexander was a pediatrician and microbiologist who made fundamental contributions in the early studies of the genetic basis of bacterial **antibiotic resistance**, specifically the resistance displayed by *Hemophilus influenzae*, the cause of influenzal **meningitis** (swelling of the nerves in the spinal cord and brain). Her pioneering studies paved the way for advances in treatment that have saved countless lives.

Alexander was born in Baltimore, Maryland. She received her B.A. degree from Goucher College in 1923. After working as a **public health** bacteriologist from 1923 to 1926, she entered the Johns Hopkins School of Medicine. She received her M.D. in 1930. Alexander assumed a residency at New York City Babies Hospital in 1930. She remained there for the remainder of her career, attaining the rank of Professor in 1957.

Alexander pioneered studies of the antibiotic resistance and susceptibility of *Hemophilus influenzae*. In 1939 she successfully utilized an anti-**pneumonia** serum that had been developed at Rockefeller University to cure infants of influenzal meningitis. Until then, infection with *Hemophilus influenzae* type b almost always resulted in death. Her **antiserum** reduced the death rate by almost 80%. Further research led to the use of **sulfa drugs** and other **antibiotics** in the treatment of the meningitis.

In other research, Alexander established that *Hemophilus influenzae* was the cause of a malady known as epiglottitis (also called croup). Her discovery prompted research that has led to effective treatments for croup.

In the 1950s Alexander began studies on the genetic basis of antibiotic resistance. During the next two decades she made fundamental observations concerning bacterial and **viral genetics**. She demonstrated that the ability of *Hemophilus influenzae* to cause disease rested with its genetic material. Additionally she demonstrated that the genetic material of poliovirus could infect human cells. She also proposed that the mechanisms of inheritance of traits in **microorganisms** could be similar to the mechanisms operating in humans. Time has borne out her suggestion.

In addition to her research, Alexander devoted much time to teaching and clinical duties. For her research and other professional accomplishments Alexander received many awards, honorary degrees, and other honors. Notably she became the first woman president of the American Pediatric Society in 1965.

See also Bacterial adaptation; Microbial genetics

ALGAE, ECONOMIC USES AND BENEFITS •

see ECONOMIC USES AND BENEFITS OF MICROORGANISMS

ALLERGIES

An allergy is an excessive or hypersensitive response of the **immune system** to harmless substances in the environment. Instead of fighting off a disease-causing foreign substance, the immune system launches a complex series of actions against an irritating substance, referred to as an allergen. The immune response may be accompanied by a number of stressful symptoms, ranging from mild to severe to life threatening. In rare cases, an allergic reaction leads to anaphylactic shock—a condition characterized by a sudden drop in blood pressure, difficulty in breathing, skin irritation, collapse, and possible death.

The immune system may produce several chemical agents that cause allergic reactions. Some of the main immune system substances responsible for the symptoms of allergy are the histamines that are produced after an exposure to an allergen. Along with other treatments and medicines, the use of antihistamines helps to relieve some of the symptoms of allergy by blocking out **histamine** receptor sites. The study of allergy medicine includes the identification of the different types of allergy, **immunology**, and the diagnosis and treatment of allergy.

The most common causes of allergy are pollens that are responsible for seasonal or allergic rhinitis. The popular name for rhinitis, hay fever, a term used since the 1830s, is inaccurate because the condition is not caused by fever and its symptoms do not include fever. Throughout the world during every season, pollens from grasses, trees, and weeds produce allergic reactions like sneezing, runny nose, swollen nasal tissues, headaches, blocked sinuses, and watery, irritated eyes. Of the 46 million allergy sufferers in the United States, about 25 million have rhinitis.

Dust and the house dust mite constitute another major cause of allergies. While the mite itself is too large to be inhaled, its feces are about the size of pollen grains and can lead to allergic rhinitis. Other types of allergy can be traced to the fur of animals and pets, food, drugs, insect bites, and skin contact with chemical substances or odors. In the United States, there are about 12 million people who are allergic to a variety of chemicals. In some cases an allergic reaction to an insect sting or a drug reaction can cause sudden death. Serious asthma attacks are sometimes associated with seasonal rhinitis and other allergies. About nine million people in the United States suffer from asthma.

Some people are allergic to a wide range of allergens, while others are allergic to only a few or none. The reasons for these differences can be found in the makeup of an individual's immune system. The immune system is the body's defense against substances that it recognizes as dangerous to the body. Lymphocytes, a type of white blood cell, fight **viruses**, **bacteria**, and other antigens by producing antibodies. When an allergen first enters the body, the lymphocytes produce an **antibody** called immunoglobulin E (IgE). The IgE antibodies attach to mast cells, large cells that are found in connective tissue and contain histamines along with a number of other chemical substances.

Studies show that allergy sufferers produce an excessive amount of IgE, indicating a hereditary factor for their allergic responses. How individuals adjust over time to allergens in their environments also determines their degree of susceptibility to allergic disorders.

The second time any given allergen enters the body, it becomes attached to the newly formed Y-shaped IgE antibodies. These antibodies, in turn, stimulate the mast cells to discharge its histamines and other anti-allergen substances. There are two types of histamine: H_1 and H_2. H_1 histamines travel to receptor sites located in the nasal passages, respiratory system, and skin, dilating smaller blood vessels and constricting airways. The H_2 histamines, which constrict the larger blood vessels, travel to the receptor sites found in the salivary and tear glands and in the stomach's mucosal lining. H_2 histamines play a role in stimulating the release of stomach acid, thus contributing to a seasonal stomach ulcer condition.

The simplest form of treatment is the avoidance of the allergic substance, but that is not always possible. In such cases, desensitization to the allergen is sometimes attempted by exposing the patient to slight amounts of the allergen at regular intervals.

Antihistamines, which are now prescribed and sold over the counter as a rhinitis remedy, were discovered in the 1940s. There are a number of different antihistamines, and they either inhibit the production of histamine or block them at receptor sites. After the administration of antihistamines, IgE receptor sites on the mast cells are blocked, thereby preventing the release of the histamines that cause the allergic reactions. The allergens are still there, but the body's "protective" actions are suspended for the period of time that the antihistamines are active. Antihistamines also constrict the smaller blood vessels and capillaries, thereby removing excess fluids. Recent research has identified specific receptor sites on the mast cells for the IgE. This knowledge makes it possible to develop medicines that will be more effective in reducing the symptoms of various allergies.

Hayfever allergy triggered by oilseed rape plants.

Corticosteroids are sometimes prescribed to allergy sufferers as anti-inflammatories. Decongestants can also bring relief, but these can be used for a short time only, since their continued use can set up a rebound effect and intensify the allergic reaction.

See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Antigenic mimicry; Immunology

AMEBIC DYSENTERY

Amebic (or amoebic) **dysentery**, which is also referred to as amebiasis or amoebiasis, is an **inflammation** of the intestine caused by the parasite *Entamoeba histolytica*. The severe form of the malady is characterized by the formation of localized lesions, called ulcers, in the intestine, especially in the region known as the colon, abscesses in the liver and the brain, and by vomiting, severe diarrhea with fluid loss leading to dehydration, and abdominal pain.

Amebic dysentery is one of the two most common causes of intestinal inflammation worldwide. The other is infection with **bacteria** of the *Shigella* group.

Amebiasis is contracted mainly by ingesting the parasite in contaminated food or water. Person–to–person transmission is less likely, but can occur. The disease is thus most common where sanitation is poor, in the developing world. The disease is especially prevalent in regions where untreated human waste is used as fertilizer. Run–off from fields can contaminate wells contaminating the drinking water. Amebiasis can occur anywhere in the world in almost any climate, excluding polar areas and mountainous high altitudes. Even now, approximately 500 cases are reported each year in New York State.

Those infected with the parasite may develop the severe symptoms listed above, a milder condition characterized by nausea, loose bowel movements and pain in the abdomen, or sometimes no symptoms at all. The latter is a concern to others, as the asymptomatic person can still pass the parasite in his/her feces and so potentially spread the infection to others. Indeed, such transmission can persist even years after exposure to the parasite.

Entamoeba histolytica can occur in two forms. The parasite is excreted to the environment as a so-called cyst from. This form is very hardy, and is analogous to a bacterial spore. This form is designed for longevity, to preserve the genetic material of the parasite when in inhospitable environments. Once in a more favorable environment, such as the intestinal tract of humans, the cyst resuscitates and growth resumes. The active and growing form of the parasite is known as a trophozoite. It is the trophozoite that causes the symptoms of amebiasis. Some trophozoites will re-encyst and exit via the feces, to become a potential source of further infection.

If the cyst stays in the intestinal tract after being ingested then they have little adverse effect. However, if the cysts invade the walls of the intestine, ulcers and diarrhea can be produced. Amebiasis can be fairly short in duration, lasting only a few weeks. Or, the infection may become chronic. The chronic form can be ominous, as the trophozoite can invade the blood and be carried all over the body. The abscesses formed in the liver and brain can be very destructive.

Both amebiasis and the causative parasite have been known for a long time. The parasite was described in great detail and given its name in 1903. Despite this long history, the diagnosis of the malady still relies on the visual detection of the parasite in fecal material obtained from a suspected patient. Often fecal samples need to be examined for several days to detect the presence of cysts. Amebiasis is still easily misdiagnosed, especially when no symptoms are present. Also the parasite can be visually similar to harmless normal residents of the intestinal tract, such as *Entamoeba coli*, and can co-exist with bacteria that themselves are the cause of the symptoms being experienced by the infected person.

Amebiasis is treatable, usually by a combination of drugs. An amebicide will kill the organisms in the intestinal tract, while an antibiotic will treat any bacteria that have been ingested with the feces, contaminated water, or food. Finally, if warranted, a drug can be administered to retard the spread of the infection to tissues such as the liver.

See also Parasites

American Type Culture Collection

The American Type Culture Collection, which is also known as the ATCC, is a not-for-profit bioscience organization that maintains the world's largest and most diverse collection of microbiological life. Many laboratories and institutions maintain their own stockpile of **microorganisms**, usually those that are in frequent use in the facility. Some large culture collections are housed and maintained, usually by universities or private enterprises. But none of these rivals the ATCC in terms of size.

Technician at The American Type Culture Collection.

The ATCC collection includes repositories of bacterial species, animal **viruses**, cell lines (which are important for the growth of certain types of viruses), **fungi**, **plant viruses**, **protists** (microscopic organisms that have a **nucleus** that is contained within a membrane), and yeasts. As well, in conjunction with researchers at George Mason University, which borders the ATCC facility, research in areas such as **bioinformatics** is carried out.

The ATCC was founded, and continues to function, to acquire, confirm the identity of, preserve and distribute biological materials to scientists worldwide. Since its inception, the mandate has expanded to now include information technology and intellectual property. Today, in addition to offering the microbiological organisms for sale, the ATCC offers technical services and educational programs to academic, government, and private organizations.

The genesis of the ATCC began in 1921. Then, the Army Medical Museum accepted a then renowned culture collection called the Winslow Culture Collection. The collection was put under the care of the Washington, D.C. members of the Society of American Bacteriologists (in time, this society grew in scope and membership to become the American Society for Microbiology). In 1925, the ATCC became an official entity with its incorporation. The burgeoning culture collection was moved to the McCormick Institute in Chicago. Twelve years later the collection returned to Washington. Space was leased to house the collection. Over the years the increasing diversification of the ATCC and the acquisition of more cultures taxed the space, so a series of moves to larger and larger sites occurred. Finally, in 1998, the organization moved to the state-of-the-art facility it continues to occupy.

The present facility is 106,000 square feet in size and has almost 35,000 square feet of laboratory space, including specialized containment facilities for more hazardous house microorganisms. Over fifty ultra-low temperature freezers are used for the long-term storage of samples. Such storage avoids changes in the organisms that could result from storage at refrigeration temperatures.

See also Cryoprotection

AMES, BRUCE N. (1928-)

American biochemist and molecular biologist

Bruce N. Ames is a professor of **biochemistry** and **molecular biology** at the University of California at Berkeley. He is best known for the development of a test used as an indicator of the carcinogenicity (cancer-causing potential) of chemicals. Known as the Ames test, it measures the rate of mutation in **bacteria** after the introduction of a test substance. Ames's research led to a greater appreciation of the role of genetic mutation in cancer and facilitated the testing of suspected cancer-causing chemicals. He also developed a database of chemicals that cause cancer in animals, listing their degree of virulence. Ames has been involved in numerous controversies involving scientific and environmental policies relevant to cancer prevention. In the 1970s he vociferously advocated strict government control of synthetic chemicals. In the 1980s, however, the discovery that many natural substances were also mutagenic (causing **gene** mutation), and thus possibly cancer causing, led him to reverse his original position.

Ames was born in New York City, the son of Dr. Maurice U. and Dorothy Andres Ames. His father taught high school science and then became assistant superintendent of schools. Ames himself graduated from the Bronx High School of Science in 1946. He received a B.A. in biochemistry from Cornell University in 1950 and a Ph.D. in the same field from the California Institute of Technology in 1953. Ames worked at the National Institutes of Health, primarily in the National Institute of Arthritis and Metabolic Diseases, from 1953 to 1967. In 1968 he moved to the Department of Biochemistry and Molecular Biology at the University of California at Berkeley as a full professor. He was Chairman of the Department from 1984 to 1989. In addition he became Director of the National Institute of Environmental Health Science at the University in 1979.

In the 1960s and early 1970s Ames developed a test that measured the degree to which synthetic chemicals cause gene mutation (a change in the **deoxyribonucleic acid**, or **DNA**, the molecule that carries genetic information). He began by deliberately mutating a *Salmonella* bacterium. The changed bacterium could not produce an amino acid called histidine that normal bacteria produce and that they need to survive. The next step was to add just enough histidine to allow the bacteria to live, and to add, as well, the synthetic chemical being tested. If the added chemical caused genetic mutation, the abnormal gene of the *Salmonella* bacteria would mutate and again be able to produce histidine. When this happened the added chemical was marked as a suspected carcinogen, because cancer is associated with somatic cell mutation (that is, mutation of any cells with the exception of germ cells).

Over eighty percent of organic chemicals known to cause cancer in humans tested positive as mutagens in the test developed by Ames and his colleagues. This result gave support to the theory that somatic mutation causes cancer and helped to validate the use of the test for initial identification of mutagens when considering synthetic chemicals for industrial and commercial use. In addition to these practical results, the research of Ames and a colleague, H. J. Whitfield, Jr., led to important advances in understanding the biochemistry of mutagenesis. Beyond his work in genetic toxicology, Ames made important discoveries in molecular biology, including ground-breaking studies on the regulation of the histidine **operon** (the gene or locus of the gene that controls histidine) and the role of transfer **ribonucleic acid** (**RNA**) in that regulation.

In the 1980s Ames set up a database of animal cancer test results with colleague Lois Swirsky Gold of Lawrence Berkeley Laboratory. The database is used to determine whether a chemical has tested positive as a carcinogen and gives the degree of its virulence. From these data Ames developed a value measuring the carcinogenic danger of a chemical to humans. HERP (daily Human Exposure dose/Rodent Potency dose) is the value determined by comparing the daily dose of a chemical that will cause cancer in half a group of test animals with the estimated daily dose to which humans are normally exposed. The result is a percentage that suggests the degree of carcinogenicity of a chemical for humans.

In the 1970s Ames was a conspicuous advocate of particular regulatory and environmental public policies that relate to the cancer-causing potential of synthetic substances. In the 1970s Ames asserted that even trace amounts of mutagenic chemicals could cause a mutation (and thus possibly cancer). He found that tris (2,3-dibromopropyl) phosphate, the chemical that was used as a flame retardant on children's pajamas, was a mutagen in the Ames test; he was instrumental in getting it banned. Similarly he found that some hair dyes contained mutagens. His advocacy led to governmental regulations that forced manufacturers to reformulate their products. In his position on the regulation of synthetic chemicals, he was a natural ally of environmentalists.

However, in the early 1980s Ames reversed his position, arguing that there is no scientific evidence that small doses of most synthetic chemicals cause human cancers; he also argued that, in the absence of such evidence, they should not be controlled. This about-face was partly a result of a growing body of knowledge concerning the mutagenic properties of numerous chemicals found in nature. Ames began arguing against the existing large public expenditures for pollution control and the regulation of synthetic chemicals, noting that cancer might just as plausibly be caused by the chemicals in plants. His arguments were based primarily on three factors: his argument that more scientific evidence should be required before controls are implemented; his attitude toward the setting of prior-

ities, which he argued should be centered on basic research rather than regulation; and finally his belief that the large public expenditures incurred by the regulatory process hurt American economic competitiveness.

Ames and his colleague Gold have also argued that the use of bioassays (animal tests) of chemicals to predict their carcinogenic potential in humans should be abandoned. In a typical bioassay, rats are given a maximum tolerated dosage (MTD) of a particular chemical daily for a period of time (such as a year). The maximum tolerated dosage is as much as the animal can be given without immediately becoming ill or dying. At the end of the time period, the number of animals that have developed cancers is tabulated as an indicator of the cancer causing potential of the chemical being tested. Ames suggested that it is often the large dosage itself, rather than the nature of the particular chemical that induces the rat cancers. He argued that, since humans are not normally exposed to such large doses, the assays were not valid for predicting human cancers.

Ames's arguments have some support both within and outside scientific communities. However, he also has numerous critics. Those taking issue with his positions have noted that pollution control, for example, involves far more than just carcinogenicity. These critics suggest that Ames has not offered a substitute for animal assays (the Ames test has not proved to be such a substitute), and that neither he nor they have a good idea of what goes on at low dosages. Some argue that Ames has an over-simplified view of the regulatory process, which is based on a consideration of animal assays but also on other factors. It has also been argued that the discovery that many naturally occurring chemicals have a high mutagenic rate (just as synthetic chemicals) should not lead to the conclusion that synthetic chemicals pose less risk than was previously supposed. Such an assumption places too much emphasis on mutagenic rate as a sole indicator of carcinogenicity, ignoring the complex, multi-stage developmental process of the disease.

Yet the disagreements between Ames and his critics are based on several points of commonality—that cancer is a complex multi-stage process that is not fully understood; that there is no perfect test or group of tests that can fully predict the potential carcinogenicity of many substances in humans; and that public regulatory and environmental policies must be made and carried out in spite of this deficiency of knowledge. As for Ames, he has described his public-policy activism as a hobby, and he has noted that his recent scientific work includes studies in the biochemistry of aging.

Elected to the National Academy of Sciences in 1972, Ames has received many awards, including the Eli Lilly Award of the American Chemical Society (1964), the Mott Prize of the General Motors Cancer Research Foundation (1983), and the Gold Medal of the American Institute of Chemists (1991). He is the author or coauthor of more than 250 scientific articles.

See also Chemical mutagenesis; Molecular biology and molecular genetics

AMINO ACID CHEMISTRY

Amino acids are the building blocks of proteins and serve many other functions in living organisms. The prime function of **DNA** is to carry the information needed to direct the proper sequential insertion of amino acids into protein chain during **protein synthesis** (**translation**).

An amino acid is a molecule that contains a terminal acidic carboxyl group (COOH) and a terminal basic amino group (NH_2). The approximately 20 amino acids (plus a few derivatives) that have been identified as protein constituents are alpha-amino acids in which the -NH_2 group is attached to the alpha-carbon next to the -COOH group. Thus, their basic structure is $NH_2CHRCOOH$, where R is a side chain. This side chain, which uniquely characterizes each alpha-amino acid, determines the molecules overall size, shape, chemical reactivity, and charge. There are hundreds of alpha-amino acids, both natural and synthetic.

The amino acids that receive the most attention are the alpha-amino acids that genes are codes for, and that are used to construct proteins. These amino acids include glycine NH_2CH_2COOH, alanine CH_3CH (NH_2) COOH, valine $(CH_3)2CHCH$ $(NH_2)COOH$, leucine $(CH_3)_2CHCH_2CH(NH_2)$ COOH, isoleucine $CH_3CH_2CH(CH_3)CH(NH_2)COOH$, methionine $CH_3SCH_2CH_2CH(NH_2)COOH$, phenylalanine $C_6H_5CH_2$ $CH(CH_2)COOH$, proline C_4H_8NCOOH, serine $HOCH_2CH$ $(NH_2)COOH$, threonine $CH_3CH(OH)CH(NH_2)COOH$, cysteine $HSCH_2CH(NH_2)COOH$, asparagine, glutamine H_2NC $(O)(CH_2)2CH(NH_2)COOH$, tyrosine $C_6H_4OHCH_2CHNH_2$ COOH, tryptophan $C_8H_6NCH_2CHNH_2COOH$, aspartate $COOHCH_2CH(NH_2)COOH$, glutamate $COOH(CH_2)2CH$ $(NH_2)COOH$, histidine $HOOCCH(NH_2)CH_2C_3H_3H_2$, lysine $NH_2(CH_2)_4CH(NH_2)COOH$, and arginine $(NH_2)C(NH)$ $HNCH_2CH_2CH_2CH(NH_2)COOH$.

Proteins are one of the most common types of molecules in living matter. There are countless members of this class of molecules. They have many functions from composing cell structure to enabling cell-to-cell communication. One thing that all proteins have in common is that they are composed of amino acids.

Proteins consist of long chains of amino acids connected by peptide linkages (-CO·NH-). A protein's primary structure refers to the sequence of amino acids in the molecule. The protein's secondary structure is the fixed arrangement of amino acids that results from interactions of amide linkages that are close to each other in the protein chain. The secondary structure is strongly influenced by the nature of the side chains, which tend to force the protein molecule into specific twists and kinks. Side chains also contribute to the protein's tertiary structure, i.e., the way the protein chain is twisted and folded. The twists and folds in the protein chain result from the attractive forces between amino acid side chains that are widely separated from each other within the chain. Some proteins are composed of two of more chains of amino acids. In these cases, each chain is referred to as a subunit. The subunits can be structurally the same, but in many cases differ. The protein's quaternary structure refers to the spatial arrangement of the subunits of the protein, and

Name	The *XYZ* group is	Shorthand
Glycine	—H	Gly
Alanine	$-CH_3$	Ala
Valine	$-CH(CH_3)_2$	Val
Leucine	$-CH_2CH(CH_3)_2$	Leu
Isoleucine	$-CH(CH_3)CH_2CH_3$	Ile
Serine	$-CH_2OH$	Ser
Threonine	$-CH(OH)CH_3$	Thr
Cysteine	$-CH_2SH$	Cys
Methionine	$-CH_2CH_2SCH_3$	Met
Aspartic acid	$-CH_2C(=O)OH$	Asp
Asparagine	$-CH_2C(=O)NH_2$	Asn
Glutamic acid	$-CH_2CH_2C(=O)OH$	Glu
Glutamine	$-CH_2CH_2C(=O)NH_2$	Gln
Lysine	$-CH_2CH_2CH_2CH_2NH_2$	Lys
Arginine	$-CH_2CH_2CH_2NHC(=NH)NH_2$	Arg
Histidine	$-CH_2C(=CH)NHCHN$	His
Phenylalanine	$-CH_2C_6H_5$	Phe
Tyrosine	$-CH_2C_6H_4OH$	Tyr
Tryptophan	$-CH_2C_8H_6N$	Trp
Proline	$HOC(=O)CHCH_2CH_2CH_2N$	Pro

The twenty most common amino acids. ***Illustrations reprinted by permission of Robert L. Wolke.***

describes how the subunits pack together to create the overall structure of the protein.

Even small changes in the primary structure of a protein may have a large effect on that protein's properties. Even a single misplaced amino acid can alter the protein's function. This situation occurs in certain genetic diseases such as sickle-cell anemia. In that disease, a single glutamic acid molecule has been replaced by a valine molecule in one of the chains of the hemoglobin molecule, the protein that carries oxygen in red blood cells and gives them their characteristic color. This seemingly small error causes the hemoglobin molecule to be misshapen and the red blood cells to be deformed. Such red blood cells cannot distribute oxygen properly, do not live as long as normal blood cells, and may cause blockages in small blood vessels.

Enzymes are large protein molecules that catalyze a broad spectrum of biochemical reactions. If even one amino acid in the enzyme is changed, the enzyme may lose its catalytic activity.

The amino acid sequence in a particular protein is determined by the protein's **genetic code**. The genetic code resides in specific lengths (called genes) of the polymer doxyribonucleic acid (DNA), which is made up of from 3000 to several million nucleotide units, including the nitrogeneous bases: adenine, guanine, cytosine, and thymine. Although there are only four nitrogenous bases in DNA, the order in which they appear transmits a great deal of information. Starting at one end of the **gene**, the genetic code is read three nucleotides at a time. Each triplet set of nucleotides corresponds to a specific amino acid.

Occasionally there an error, or mutation, may occur in the genetic code. This mutation may correspond to the substitution of one nucleotide for another or to the deletion of a nucleotide. In the case of a substitution, the result may be that the wrong amino acid is used to build the protein. Such a mistake, as demonstrated by sickle cell anemia, may have grave consequences. In the case of a deletion, the protein may be lose its functionality or may be completely missing.

Amino acids are also the core construction materials for neurotransmitters and hormones. Neurotransmitters are chemicals that allow nerve cells to communicate with one another and to convey information through the nervous system. Hormones also serve a communication purpose. These chemicals are produced by glands and trigger metabolic processes throughout the body. Plants also produce hormones.

Important neurotransmitters that are created from amino acids include serotonin and gamma-aminobutyric acid. Serotonin($C_{10}H_{12}N_2O$) is manufactured from tryptophan, and gamma-aminobutyric acid ($H_2N(CH_2)_3COOH$) is made from glutamic acid. Hormones that require amino acids for starting materials include thyroxine (the hormone produced by the thyroid gland), and auxin (a hormone produced by plants). Thyroxine is made from tyrosine, and auxin is constructed from tryptophan.

A class of chemicals important for both neurotransmitter and hormone construction are the catecholamines. The amino acids tyrosine and phenylalanine are the building materials for catecholamines, which are used as source material for both neurotransmitters and for hormones.

Amino acids also play a central role in the **immune system**. Allergic reactions involve the release of **histamine**, a chemical that triggers **inflammation** and swelling. Histamine is a close chemical cousin to the amino acid histidine, from which it is manufactured.

Melatonin, the chemical that helps regulate sleep cycles, and melanin, the one that determines the color of the skin, are both based on amino acids. Although the names are similar, the activities and component parts of these compounds are quite different. Melatonin uses tryptophan as its main building block, and melanin is formed from tyrosine. An individual's melanin production depends both on genetic and environmental factors.

Proteins in the diet contain amino acids that are used within the body to construct new proteins. Although the body also has the ability to manufacture certain amino acids, other amino acids cannot be manufactured in the body and must be gained through diet. Such amino acids are called the essential dietary amino acids, and include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

Foods such as meat, fish, and poultry contain all of the essential dietary amino acids. Foods such as fruits, vegetables, grains, and beans contain protein, but they may lack one or more of the essential dietary amino acids. However, they do not all lack the same essential dietary amino acid. For example, corn lacks lysine and tryptophan, but these amino acids can be found in soy beans. Therefore, vegetarians can meet their dietary needs for amino acids as long by eating a variety of foods.

Amino acids are not stockpiled in the body, so it is necessary to obtain a constant supply through diet. A well-balanced diet delivers more protein than most people need. In fact, amino acid and protein supplements are unnecessary for most people, including athletes and other very active individuals. If more amino acids are consumed than the body needs, they will be converted to fat or metabolized and excreted in the urine.

However, it is vital that all essential amino acids be present in the diet if an organism is to remain healthy. Nearly all proteins in the body require all of the essential amino acids in their synthesis. If even one amino acid is missing, the protein cannot be constructed. In cases in which there is an ongoing deficiency of one or more essential amino acids, an individual may develop a condition known as kwashiorkor, which is characterized by severe weight loss, stunted growth, and swelling in the body's tissues. The situation is made even more grave because the intestines lose their ability to extract nutrients from whatever food is consumed. Children are more strongly affected by kwashiorkor than adults because they are still growing and their protein requirements are higher. Kwashiorkor often accompanies conditions of famine and starvation.

See also Bacterial growth and division; Biochemistry; Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Cell cycle and cell division; Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); Enzymes; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Genotype and phenotype; Molecular biology and molecular genetics

AMINOGLYCOSIDE ANTIBIOTICS • *see* ANTIBIOTICS

AMYLOID PLAQUES • *see* BSE AND CJD DISEASE

ANAEROBES AND ANAEROBIC INFECTIONS

Anaerobes are **bacteria** that are either capable of growing in the absence of oxygen (referred to as facultative anaerobes) or that absolutely require the absence of oxygen (these are also called obligate anaerobes). Among the oxygen-free environments in which such bacteria can grow are deep wounds and tissues in the body. Growth in these niches can produce infections.

Examples of infections are gas gangrene (which is caused by *Streptococcus pyogenes*) and **botulism** (which is caused by *Clostridium botulinum*). Other anaerobic bacteria that are frequently the cause of clinical infections are members of the genus *Peptostreptococcus* and *Bacteroides fragilis*.

There are a number of different types of anaerobic bacteria. Two fundamental means of differentiation of these types is by their reaction to the Gram stain and by their shape. The genus *Clostridium* consists of Gram-positive rod-shaped bacteria that form spores. Gram-positive rods that do not form spores include the genera *Actinomyces*, *Bifidobacterium*, *Eubacterium*, *Propionibacterium*, and *Lactobacillus*. Gram-positive bacteria that are spherical in shape includes the gen-

era *Peptostreptococcus*, *Streptococcus*, and *Staphylococcus*. Rod-shaped bacteria that stain Gram-negative include *Bacteroides*, *Campylobacter*, and *Fusobacterium*. Finally, Gram-negative spherical bacteria are represented by the genus *Veillonella*.

The word anaerobic means "life without air." In the human body, regions that can be devoid of oxygen include the interior of dental **plaque** that grows on the surface of teeth and gums, the gastrointestinal tract, and even on the surface of the skin. Normally the anaerobic bacteria growing in these environments are benign and can even contribute to the body's operation. Most of the bacteria in the body are anaerobes. However, if access to underlying tissues is provided due to injury or surgery, the bacteria can invade the new territory and establish an infection. Such bacteria are described as being opportunistic pathogens. That is, given the opportunity and the appropriate conditions, they are capable of causing an infection. Typically, anaerobic bacteria cause from five to ten per cent of all clinical infections.

Anaerobic infections tend to have several features in common. The infection is usually accompanied by a foul-smelling gas or pus. The infections tend to be located close to membranes, particularly mucosal membranes, as the infection typically begins by the invasion of a region that is bounded by a membrane. Anaerobic infections tend to involve the destruction of tissue, either because of bacterial digestion or because of destructive **enzymes** that are elaborated by the bacteria. This type of tissue damage is known as tissue necrosis. The tissue damage also frequently includes the production of gas or a fluid.

There are several sites in the body that are prone to infection by anaerobic bacteria. Infections in the abdomen can produce the **inflammation** of the appendix that is known as appendicitis. Lung infections can result in **pneumonia**, infection of the lining of the lung (empyema) or constriction of the small air tubes known as bronchi (bronchiectasis). In females, pelvic infections can inflame the lining of the uterus (endometritis). Mouth infections can involve the root canals or gums (gingivitis). Infections of the central nervous system can lead to brain and spinal cord infections. Infection of the skin, via bites and other routes of entry, causes open sores on the skin and tissue destruction. An example is that massive and potentially lethal tissue degradation, which is known as necrotizing fascitis, and which is caused by group A b-hemolytic *Streptococcus*. Finally, infection of the bloodstream (bacteremia) can prelude the infection of the heart (endocarditis).

The diagnosis of anaerobic infections is usually based on the symptoms, site of the infection and, if the infection is visible, on both the appearance and smell of the infected area. Most of the bacteria responsible for infection are susceptible to one or more **antibiotics**. Treatment can be prolonged, however, since the bacteria are often growing slowly and since antibiotics rely on **bacterial growth** to exert their lethal effect. In the case of infections that create tissue destruction, the removal of the affected tissue is an option to prevent the spread of the infection. Amputation of limbs is a frequent means of dealing with necrotizing fascitis, an infection that is inside of tissue (and so protected from antibiotics and the host's immune response) and is exceptional in that it can swiftly spread.

See also Bacteria and bacterial infections

ANAPHYLACTIC SHOCK • *see* IMMUNITY: ACTIVE, PASSIVE, AND DELAYED

ANAPHYLAXIS

Anaphylaxis is a severe allergic reaction. The symptoms appear rapidly and can be life threatening.

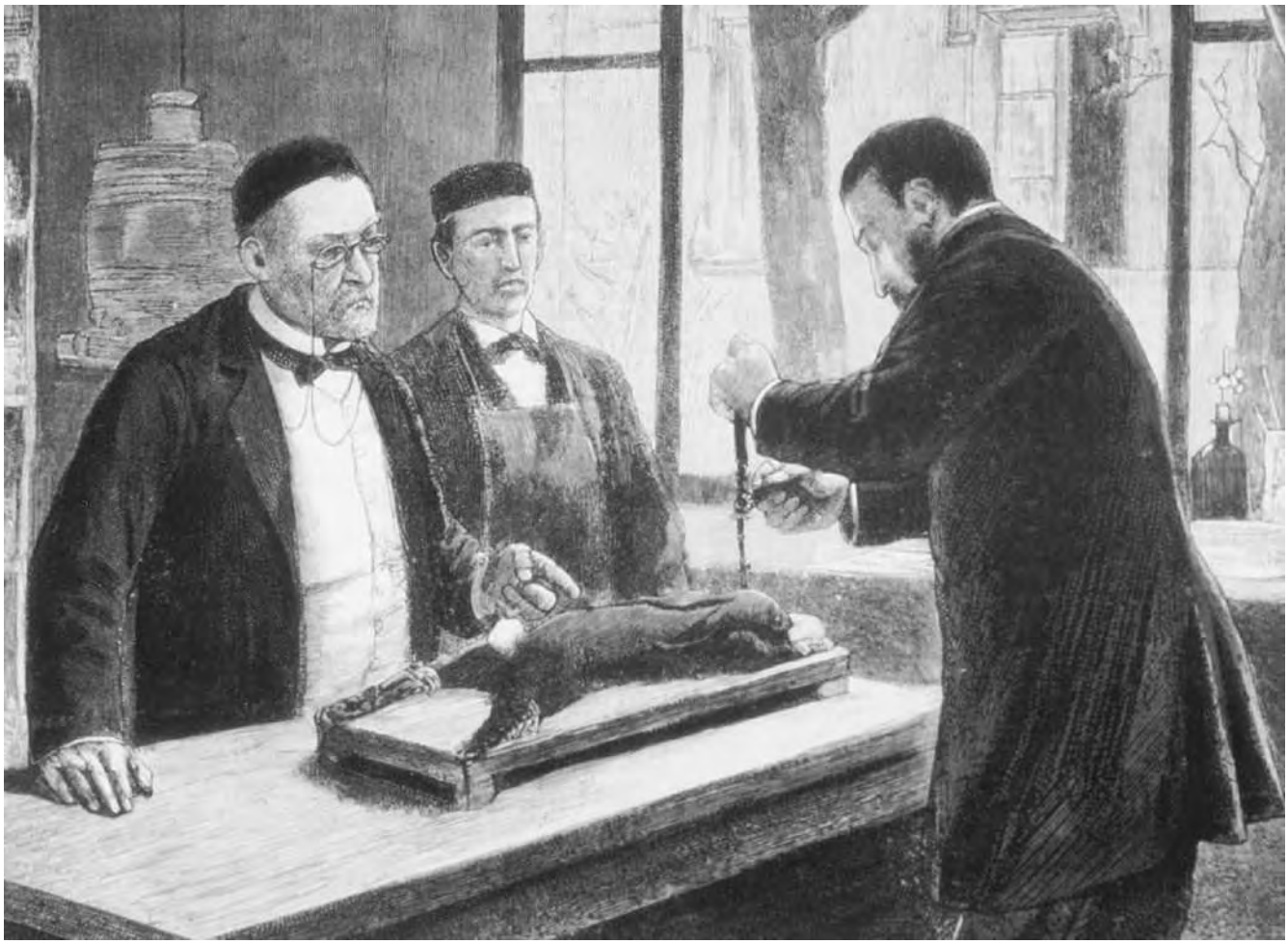
The symptoms of anaphylaxis include the increased output of fluid from mucous membranes (e.g., passages lining the nose, mouth, and throat), skin rash (e.g., hives), itching of the eyes, gastrointestinal cramping, and stiffening of the muscles lining the throat and trachea. As a result of the latter, breathing can become difficult. These symptoms do not appear in every case. However, some sort of skin reaction is nearly always evident.

Anaphylaxis results from the exposure to an **antigen** with which the individual has had previous contact, and has developed a heightened sensitivity to the antigen. Such an antigen is also known as an allergen. The allergen binds to the specific immune cell (e.g., immunoglobulin E, also known as IgE) that was formed in response to the initial antigen exposure. IgE is also associated with other specific cells of the **immune system** that are called basophils and mast cells. The basophils and mast cells react to the binding of the allergen-IgE complex by releasing compounds that are known as mediators (e.g, **histamine**, prostaglandin D2, trypase). Release of mediators does not occur when IgE alone binds to the basophils or mast cells.

The release of the mediators triggers the physiological reactions. For example, blood vessels dilate (become larger in diameter) and fluid can pass across the blood vessel wall more easily. Because the immune system is sensitized to the particular allergen, and because of the potent effect of mediators, the development of symptoms can be sudden and severe. A condition called anaphylactic shock can ensue, in which the body's physiology is so altered that failure of functions such as the circulatory system and breathing can occur. For example, in those who are susceptible, a bee sting, administration of a penicillin-type of antibiotic, or the ingestion of peanuts can trigger symptoms that can be fatal if not addressed immediately. Those who are allergic to bee stings often carry medication with them on hikes.

Anaphylaxis occurs with equal frequency in males and females. No racial predisposition towards anaphylaxis is known. The exact number of cases is unknown, because many cases of anaphylaxis are mistaken for other conditions (e.g., food poisoning). However, at least 100 people die annually in the United States from anaphylactic shock.

See also Allergies; Immunoglobulins and immunoglobulin deficiency syndromes



Drawing depicting Louis Pasteur (right) using an animal model.

ANIMAL MODELS OF INFECTION

The use of various animals as models for microbiological infections has been a fundamental part of infectious disease research for more than a century. Now, techniques of genetic alteration and manipulation have made possible the design of animals so as to be specifically applicable to the study of a myriad of diseases.

The intent for the use of animals as models of disease is to establish an infection that mimics that seen in the species of concern, usually humans. By duplicating the infection, the reasons for the establishment of the infection can be researched. Ultimately, the goal is to seek means by which the infection can be thwarted. Development of a **vaccine** to the particular infection is an example of the successful use of animals in infectious disease research.

The development of the idea that maladies could be caused by **bacterial infection** grow from animals studies by **Louis Pasteur** in the mid-nineteenth century. The use of animals as models of cholera and **anthrax** enabled Pasteur to develop vaccines against these diseases. Such work would not have been possible without the use of animals.

Subsequent to Pasteur, the use of animal models for a myriad of bacterial and viral diseases has led to the production of vaccines to diseases such as **diphtheria**, **rabies**, **tuberculosis**, **poliomyelitis**, **measles**, and rubella.

Animal models are also used to screen candidate drugs for their performance in eliminating the infection of concern and also to evaluate adverse effects of the drugs. While some of this work may be amenable to study using cells grown on in the laboratory, and by the use of sophisticated computer models that can make predictions about the effect of a treatment, most scientists argue that the bulk of drug evaluation still requires a living subject.

A key to developing an animal model is the selection of an animal whose physiology, reaction to an infection, and the nature of the infection itself all mirror as closely as possible the situation in humans. The study of an infection that bears no resemblance to that found in a human would be fruitless, in terms of developing treatment strategies for the human condition.

The need to mirror the human situation has led to the development of animal models that are specifically tailored for certain diseases. One example is the so-called nude mouse, which derives its name from the fact that it has no hair. Nude mice lack a thymus, and so are immunodeficient in a number of ways. Use of nude mice has been very useful in the study of **immunodeficiency** diseases in humans, such as acquired immunodeficiency syndrome. As well, this animal model lends itself to the study of opportunistic bacterial infections, which typically occur in humans whose **immune systems** are compromised.

Depending on the infection and the focus of study, other animals have proven to be useful in infectious disease research. These animals include the rabbit, rat, guinea pig, pig, dog, and monkey. The latter in particular has been utilized in the study of **AIDS**, as primates are the genetically closest relatives to humans.

The advent of molecular techniques of genetic alteration has made the development of genetically tailored animal models possible. Thus, for example, mouse models exist in which the activity of certain genes has been curtailed. These are known as transgenic animals. The involvement of the **gene** product in the infectious process is possible on a scale not possible without the use of the animal.

The data from animal models provides a means of indicating the potential of a treatment. Furthermore, if a disease in an animal does not exactly mimic the human's condition, for example cystic fibrosis in mice, the use of the animal model provides a guide towards establishing the optimal treatment in humans. In other words, the animal model can help screen and eliminate the undesirable treatments, narrowing the successful candidates for use in human studies. Further study, involving humans, is always necessary before something such as a vaccine can be introduced for general use. Such human studies are subject to rigorous control.

The use of animals in research has long been a contentious issue, mainly due to questions of ethical treatment. This climate has spawned much legislation concerning the treatment of research animals. As well, in most institutions, an evaluation committee must approve the use of animals. If the research can be accomplished in some other way than through the use of living animals, then approval for the animal study is typically denied.

See also AIDS, recent advances in research and treatment; Giardia and giardiasis; Immunodeficiency

ANIMALCULES • *see* HISTORY OF MICROBIOLOGY

ANTHRAX

Anthrax refers to a pulmonary disease that is caused by the bacterium *Bacillus anthracis*. This disease has been present since antiquity. It may be the sooty "morain" in the Book of *Exodus*, and is probably the "burning wind of plague" that begins Homer's *Iliad*. Accounts by the Huns during their sweep across Eurasia in 80 A.D. describe mass deaths among their horse and cattle attributed to anthrax. These animals, along with sheep, are the primary targets of anthrax. Indeed, loss to European livestock in the eighteenth and nineteenth centuries stimulated the search for a cure. In 1876, **Robert Koch** identified the causative agent of anthrax.

The use of anthrax as a weapon is not a new phenomenon. In ancient times, diseased bodies were used to poison wells, and were catapulted into cities under siege. In modern times, research into the use of anthrax as a weapon was carried out during World Wars I and II. In World War II, Japanese and German prisoners were subjects of medical research, including their susceptibility to anthrax. Allied efforts in Canada, the U.S. and Britain to develop anthrax-based weapons were also active. Britain actually produced five million anthrax cakes at the Porton Down facility, to be dropped on Germany to infect the food chain.

In non-deliberate settings, humans acquire anthrax from exposure to the natural reservoirs of the microorganism; livestock such as sheep or cattle or wild animals. Anthrax has been acquired by workers engaged in shearing sheep, for example.

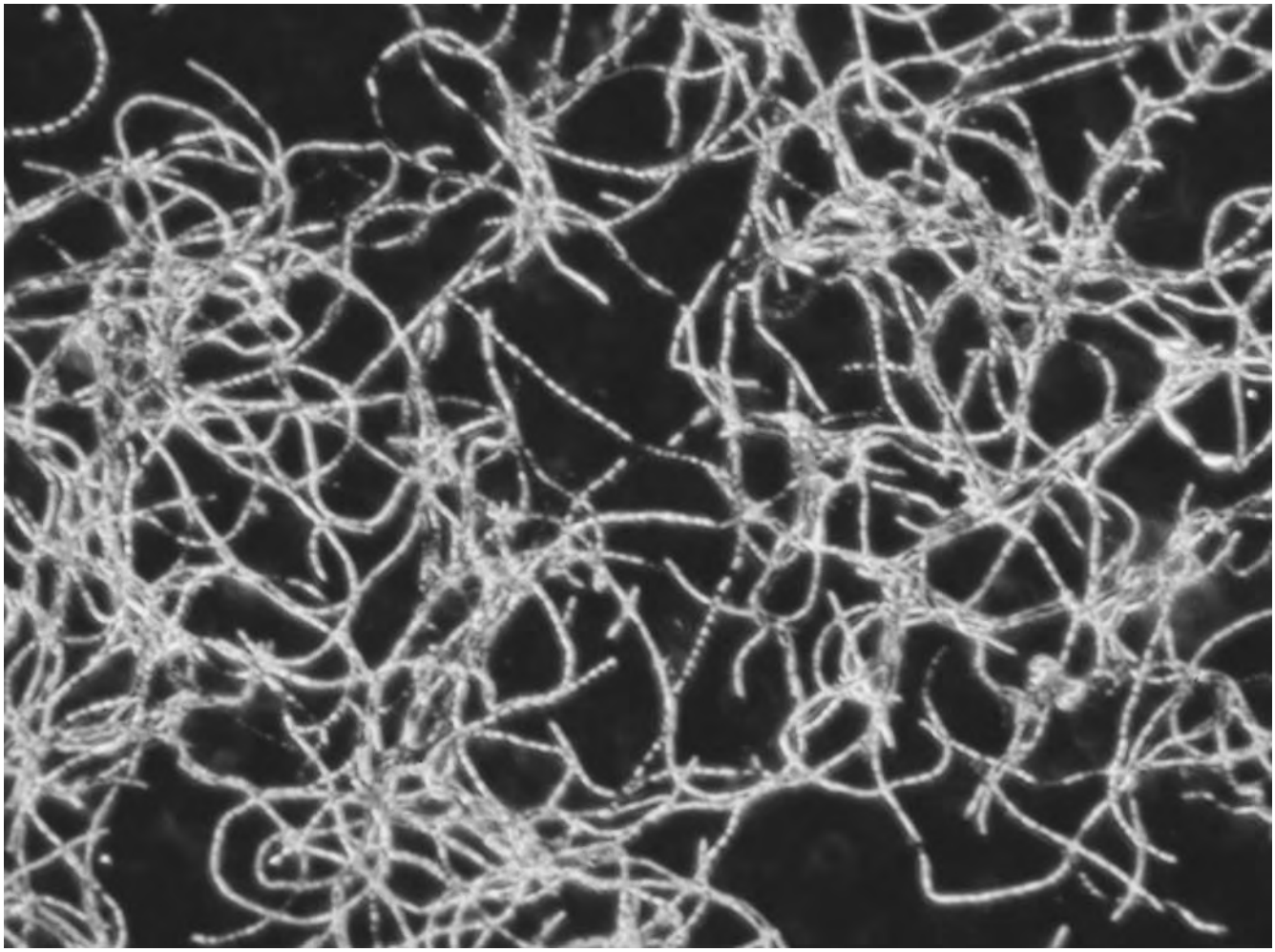
Human anthrax can occur in three major forms. Cutaneous anthrax refers to the entry of the organism through a cut in the skin. Gastrointestinal anthrax occurs when the organism is ingested in food or water. Finally, inhalation anthrax occurs when the organism is inhaled.

All three forms of the infection are serious, even lethal, if not treated. With prompt treatment, the cutaneous form is often cured. Gastrointestinal anthrax, however, can still be lethal in 25–75% of people who contract it. Inhalation anthrax is almost always fatal.

The inhalation form of anthrax can occur because of the changing state of the organism. *Bacillus anthracis* can live as a large "vegetative" cell, which undergoes cycles of growth and division. Or, the bacterium can wait out the nutritionally bad times by forming a spore and becoming dormant. The spore is designed to protect the genetic material of the bacterium during hibernation. When conditions are conducive for growth and reproduction the spore resuscitates and active life goes on again. The spore form can be easily inhaled. Only 8,000 spores, hardly enough to cover a snowflake, are sufficient to cause the pulmonary disease when they resuscitate in the warm and humid conditions deep within the lung.

The dangers of an airborne release of anthrax spores is well known. British open-air testing of anthrax weapons in 1941 on Gruinard Island in Scotland rendered the island uninhabitable for five decades. In 1979, an accidental release of a minute quantity of anthrax spores occurred at a bioweapons facility near the Russian city of Sverdlovsk. At least 77 people were sickened and 66 died. All the affected were some four kilometers downwind of the facility. Sheep and cattle up to 50 kilometers downwind became ill.

Three components of *Bacillus anthracis* are the cause of anthrax. First, the bacterium can form a capsule around itself. The capsule helps shield the bacterium from being recognized by the body's **immune system** as an invader, and helps fend off antibodies and immune cells that do try to deal



Light micrograph of *Bacillus anthracis*, showing the typical hair-like pattern of growth in a liquid.

with the bacterium. This can allow the organism to multiply to large numbers that overwhelm the immune system. The capsule also contains an **antigen** that has been designated a protective antigen. The antigen is protective, not to the host being infected, but to the bacterium. The protective antigen dissolves protein, which can allow the bacterium to "punch" through the membrane surrounding cells of the host, such as the epithelial cells that line the lung. One inside the cells, a bacterium is safe from the host's immune defenses. A second toxic component, which is called lethal factor, destroys immune cells of the host. Finally, a third toxic factor is known as edema factor (named because it results in the accumulation of fluid at the site of infection). Edema factor disables a molecule in the host called calmodulin, which is used to regulate many chemical reactions in the body. The end result of the activity of the toxic factors of *Bacillus anthracis* is to quell the immune response and so, to allow the infection to spread.

As the **bacteria** gain a foothold, toxins enter the bloodstream and circulate throughout the body causing destruction of blood cells and tissues. The damage can prove to be overwhelming to treatment efforts and death occurs.

Anthrax infections are difficult to treat because the initial symptoms are similar to other, less serious infections, such as the flu. By the time the diagnosis is made, the infection can be too advanced to treat. A **vaccine** for anthrax does exist. But to date, only those at high risk for infection (soldiers, workers in meat processing plants, anthrax research scientists) have received the vaccine, due to the possible serious side effects that can occur. Work to establish a safer vaccine is underway. The edema factor may be a potential target of a vaccine. Another promising target is the protective antigen of the capsule. If the action of this antigen could be blocked, the bacteria would not be able to hide inside host cells, and so could be more effectively dealt with by the immune response and with **antibiotics**.

See also Anthrax, terrorist use of as a biological weapon; Bioterrorism

ANTHRAX, FORENSIC IDENTIFICATION ·

see GENETIC IDENTIFICATION OF MICROORGANISMS

ANTHRAX, TERRORIST USE AS A BIOLOGICAL WEAPON

During the past two decades, the potential use of biological weapons by terrorists has received a great deal of attention, particularly in the United States. The existence of an **anthrax** bioweapons development campaign by the government of Iraq was revealed during the Persian Gulf War from 1990 to 1991. Then, in the aftermath of the September 11, 2001 terrorist attacks on the World Trade Center buildings in New York City and the Pentagon in Washington, DC., letters containing a powdered form of *Bacillus anthracis*, the **bacteria** that causes anthrax, were mailed to government representatives, members of the news media, and others in the United States. The anthrax-laced powder inside the letters was aerosolized (i.e., the spores became airborne) when the letters were opened, and in a few cases were inhaled. The death of a Florida man was the first case of an inhalational anthrax death in the United States since 1978 and as of June 2002, more than 20 cases and five deaths were attributed to the terrorist attack.

Although a relatively new weapon in the hands of modern potential bioterrorists, the threat of death from the inhalation of anthrax has been part of human history since antiquity. Some scholars argue that it is the sooty "morain" in the Bible's Book of *Exodus*, and is likely the "burning wind of plague" that begins Homer's *Iliad*.

As well, the use of **microorganisms** such as the anthrax bacteria as weapons is not new. In ancient military campaigns, diseased bodies (including those who died of anthrax) were used to poison wells and were catapulted into cities under siege. Research into the military use of anthrax was carried out during World War I by combatants on all sides of the conflict, and by World War II anthrax research was actively underway. For example, Allied efforts in Canada, the United States, and Britain to develop anthrax-based weapons included the production of five million anthrax "cakes," designed to be dropped on Germany to infect wells and the food chain. The weapons were never used.

Only within the past several decades, however, have biological weapons, including anthrax, been added to the arsenal of terrorists. For example, the Japanese cult Aum Shinrikyo (which released sarin gas into the Tokyo subway system in 1995, killing 12 people and hospitalizing 5,000) was developing anthrax-based weapons. Indeed, the group had released crude anthrax preparations in Tokyo on at least eight separate occasions in 1993. These incidents were the first time that anthrax was used as a weapon against a civilian population. In addition, state-sanctioned terrorism by the government of Iraq has also, purportedly, involved the production of anthrax bioweapons, and Western intelligence sources insist that Iraq—or terrorist groups operating with Iraq's assistance—continues (despite United Nations' efforts at inspection and destruction) to develop biological weapons, including anthrax-based weapons. Finally, during the terrorist attacks of the United States in the latter part of 2001 the use of anthrax by a terrorist or terrorists (as of June 2002, yet unidentified) pointed out how easily the lethal agent could be delivered.

This ease of delivery of anthrax is one feature that has made the bacterium an attractive weapon for terrorists. Scenarios developed by United States government agencies have shown that even a small crop dusting plane carrying only a hundred kilograms of anthrax spores flying over a city could deliver a potentially fatal dose to up to three million people in only a few hours. Although variations in weather patterns and concentration variables would substantially reduce the number of expected actual deaths, such an attack could still result in the deaths of thousands of victims and result in a devastating attack on the medical and economic infrastructure of the city attacked. In a less sophisticated effort, spores could simply be released into air intake vents or left in places like a subway tunnel, to be dispersed in the air over a much small area.

Another feature of anthrax that has led to its exploitation by terrorists is the physiology of the bacterium. *Bacillus anthracis* can live as a vegetative cell, growing and dividing in a rapid and cyclical fashion. The bacterium can also form a metabolically near-dormant form known as a spore. An individual spore is much smaller and lighter than the growing bacterium. Indeed, the spores can drift on air currents, to be inhaled into the lungs. Once in the lungs, the spores can resuscitate into an actively growing and dividing bacterium. The infections that are collectively termed anthrax can result. Although millions of spores can be released from a few grams (fractions of an ounce) of *Bacillus anthracis*, only about 5,000 to 8,000 spores are sufficient to cause the lung infection when they are inhaled. If left untreated or not promptly treated with the proper **antibiotics** (e.g., Cipro), the lung infection is almost always fatal. Non-inhalation contact with *Bacillus anthracis* can result in cutaneous anthrax—a condition more treatable with conventional antibiotic therapy.

An often-overlooked aspect of the use of anthrax as a terrorist weapon is the economic hardship that the dispersal of a small amount of the spores would exact. A report from the **Centers for Disease Control** and Prevention, entitled *The Economic Impact of a Bioterrorist Attack*, estimated the costs of dealing with an anthrax incident at a minimum of US$26 billion per 100,000 people. In just a few months in 2001 alone, a flurry of anthrax incidents, most of which turned out to be hoaxes, cost the United States government millions of dollars.

The choice of anthrax as a weapon by terrorists reflects the growing awareness of the power of biological research and **biotechnology** among the general community. The ability to grow and disperse infectious microorganisms was once restricted to specialists. However, the explosion of biotechnology in the 1980s and 1990s demonstrated that the many basic microbiological techniques are fairly simple and attainable. Experts in microbiology testifying before Congress, estimated that crude weapons could be developed with approximately $10,000 worth of equipment. A laboratory sufficient to grow and harvest the bacteria and to dry down the material to powdered form could fit into the average sized household base-

Workers in biohazard protective suits respond to an anthrax incident in Florida.

ment. The more highly trained the terrorist, the more effective weapons could be expected to be produced.

Even though *Bacillus anthracis* could be grown in such a makeshift laboratory, the preparation of the spores and the drying of the spores into a powder is not a trivial task. As an example, even after a decade of dedicated effort, United Nations inspectors who toured Iraq bioweapons facilities after the Gulf War found that Iraq had only managed to develop crude anthrax preparations. Still, the Iraq bioweapons program managed to produce 8,500 liters of concentrated anthrax.

Regardless, despite the technical challenges, the production of anthrax spores in quantities great enough to cause a huge loss of life is not beyond the capability of a small group of equipped and funded terrorists. This small size and nondescript nature of a bioweapons facility could make detection of such a lab very difficult. Accordingly, the terrorist potential of anthrax will remain a threat for the foreseeable future.

See also Bacteria and bacterial infection; Biological warfare; Bioterrorism, protective measures; Bioterrorism; Epidemics and pandemics; Vaccine

ANTI-ADHESION METHODS

The adhesion of **bacteria** and other **microorganisms** to nonliving and living surfaces is a crucial part of the **contamination** and infection processes. In fact, the growth of microorganisms on surfaces is the preferred mode of existence. The ability to block adhesion would prevent surface growth.

There are numerous examples of surface growth of microorganisms. Adherence and growth of bacteria such as **Escherichia coli** on urinary catheters (synthetic tubes that are inserted into the bladder to assist hospitalized patients in removing urine from the body) is a large problem in hospitals. The chance of a urinary tract infection increases by up to10% for each day of catheterization. *Neiserria meningitidis*, the agent that causes **meningitis**, relies upon adhesion with host cells. The adhesion of this and many other bacteria, including disease causing *Escherichia coli*, is mediated by a surface tube-like protein appendage called a pilus.

Other bacterial proteins are involved in adhesion, typically by recognizing and biding to another protein on the surface of the host cell. Microorganism proteins that function in adhesion are generically known as adhesins.

Some strains of *E. coli* that infect intestinal cells do so by manufacturing and then releasing an adhesin, which is incorporated into the membrane of the host cell. Thus, the bacteria install their own receptor in the host tissue.

Adhesion need not rely on the presence of adhesins. The chemistry of the surface can also drive adhesion. For example, the surface of the spores of bacillus and the capsule surrounding *Pasteurella multocida* are described as being **hydrophobic**; that is, they tend not to associate with water. This hydrophobicity will drive the spore or bacterium to associate with a surface of similar chemistry.

In order to block adhesion that is the result of the above mechanisms, the molecular details of these mechanisms must be unraveled. This is an on-going process, but advances are being made through research.

Adhesion of *Escherichia coli* can depend on the presence of an adhesin called FimH. Antibodies to FimH can block adhesion, presumable by binding to the FimH protein, preventing that protein from binding to the receptor on the surface of the host cell. Furthermore, the three-dimensional structure of this adhesion is similar to that of adhesins from other bacteria. A **vaccine** devised against FimH might then have some protective effect against the adhesion of other bacteria.

In the case of the capsule-mediated adhesion, such as the example above, capsular antibodies may also thwart adhesion. The drawback with this approach is that capsular material is not a potent stimulator of the **immune system**.

For microorganisms that secrete their own receptor, such as *Escherichia coli*, or which have receptor molecules protruding from their own surface (an example is the **hemagglutinin** protein on the surface of *Bordetella pertussis*), adhesion could be eliminated by blocking the manufacture or the release of the receptor molecule.

In Canada, field trials began in the summer of 2001 on a vaccine to the adhesin target of *Escherichia coli O157:H7*. This pathogen, which can be permanently debilitating and even lethal to humans who ingest contaminated food or water, often lives in the intestinal tracts of cattle. By eliminating the adhesion of the bacteria, they could be "flushed" out of the cattle. Thus, a vital reservoir of infection would have been overcome. The vaccine could be ready for the market by as early as 2003.

Another anti-adhesion strategy is to out-compete the target bacteria for the available spots on the surface. This approach has been successful in preventing bacterial vaginal infections. Suppositories loaded with bacteria called **Lactobacillus** are administered. Colonization of the vaginal wall by the Lactobacillus can retard or even prevent the subsequent colonization of the wall by a harmful type of bacteria. The same bacteria are present in yogurt. Indeed, consumption of yogurt may help prevent intestinal upset due to colonization of the gut by harmful organisms.

Non-living surfaces, such as catheters and other implanted material, are colonized by, in particular, bacteria. In seeking to prevent adhesion, scientists have been experimenting with different implant materials, with the incorporation of antimicrobial compounds into the implant material, and with the "pre-coating" of the material. In the case of antimicrobial compounds, promising results have been obtained in laboratory studies using material that can slowly release **antibiotics**. The disadvantage of this approach is that the presence of residual antibiotic could encourage the formation of resistance. Pre-coating implant material with an antimicrobial compound that is permanently bonded has also been promising in lab studies.

See also Biofilm formation and dynamic behavior; Infection and resistance; Probiotics

ANTIBIOTIC RESISTANCE, TESTS FOR

Bacteria can sometimes adapt to the **antibiotics** used to kill them. This adaptation, which can involve structural changes or the production of **enzymes** that render the antibiotic useless, can make the particular bacterial species resistant to the particular antibiotic. Furthermore, a given bacterial species will usually display a spectrum of susceptibilities to antibiotics, with some antibiotics being very effective and others totally ineffective. For another bacterial species, the pattern of antibiotic sensitivity and resistance will be different. Thus, for diagnosis of an infection and for clinical decisions regarding the best treatment, tests of an organism's response to antibiotics are essential.

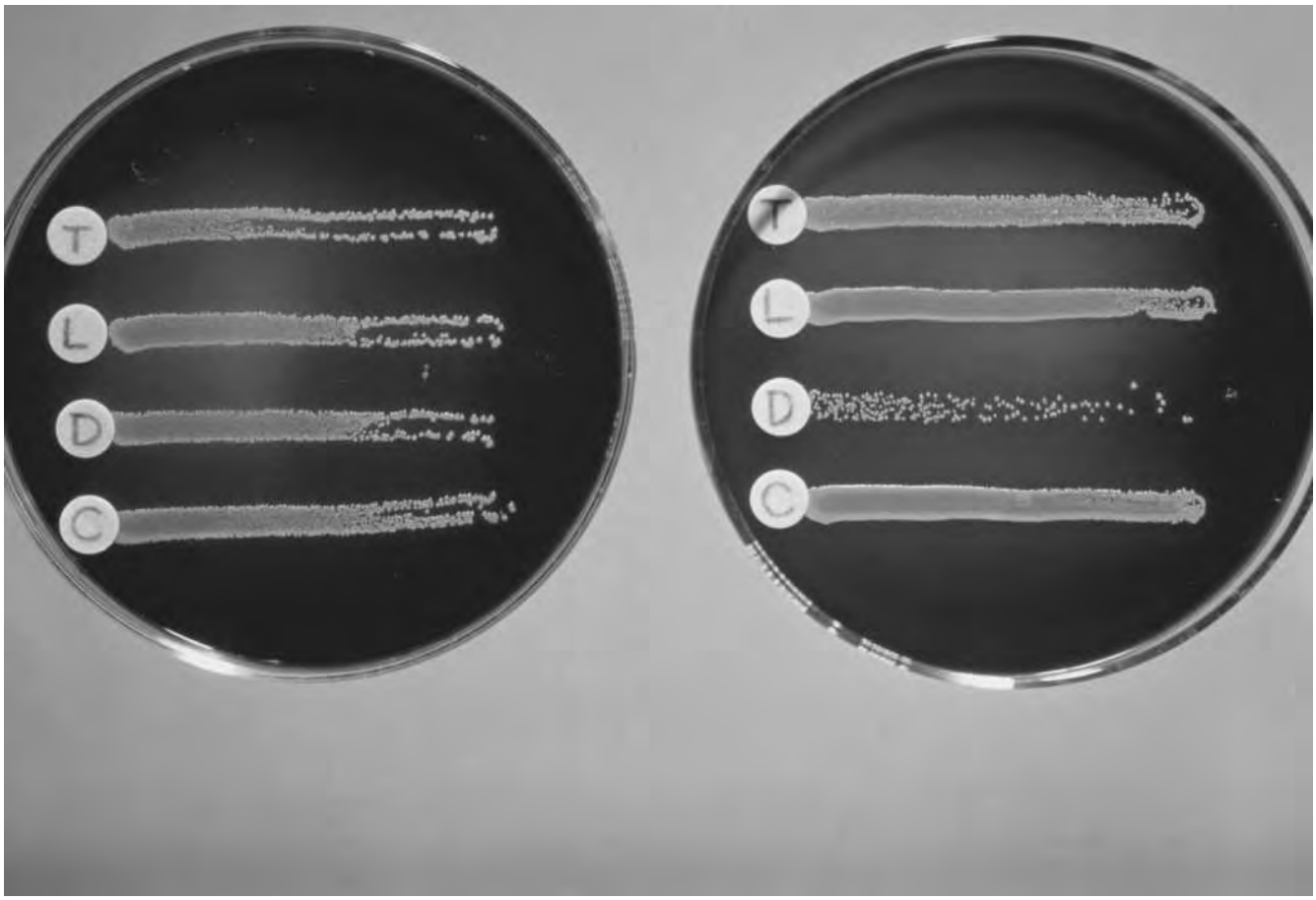
A standard method of testing for antibiotic resistance involves growth of the target bacteria in the presence of various concentrations of the antibiotic of interest. Typically, this test is performed in a specially designed plastic dish that can be filled with **agar** (a Petri plate). **Contamination** of the agar, which would spoil the test results, is guaranteed by the sterility of the plate and the lid that fits over the agar-containing dish. The type of agar used is essential for the validity of the tests results. Typically, Iso-Sensitest agar is used.

The hardened agar surface receives a suspension of the test bacteria, which is then spread out evenly over the surface of the agar. The intention is to form a so-called lawn of organisms as growth occurs. Also on the agar surface are discs of an absorbent material. A plate is large enough to house six discs. Each disc has been soaked in a known and different concentration of the same or of different antibiotics.

As growth of the bacteria occurs, antibiotic diffuses out from each disc into the agar. If the concentration of the antibiotic is lethal, no growth of the bacteria will occur. Finally, the diffusing antibiotic will be below lethal concentration, so that growth of bacteria can occur. The result is a ring of no growth around a disc. From comparison with known standards, the diameter of the growth inhibition ring will indicate whether the bacteria are resistant to the antibiotic.

Automated plate readers are available that will scan the plates, measure the diameter of the growth inhibition zones and consult a standard database to indicate the antibiotic resistance or susceptibility of the sample bacteria.

In the past 15 years, the use of fluorescent indicators has become popular. A myriad of compounds are available that will fluoresce under illumination of specific wavelengths. Among the uses for the fluorescent compounds is the viability



Antibiotic susceptible and resistant strains of Stapylococcus.

of a bacterium. For example, living bacteria will fluoresce in the presence of **acridine orange**, while dead bacteria will not. These probes combined with the optical technique of confocal laser microscopy, now enables populations of cells to be viewed without disrupting them to see if they fluoresce or not in the presence of an antibiotic of interest.

The ability of living bacteria to fluoresce can also be exploited by another machine called a flow cytometer. This machine operates essentially by forcing a suspension of bacteria (or other cells) through an opening so that only one bacterium at a time passes by a sensor. The sensor monitors each passing bacterium and can sort these into categories, in this case, fluorescing (living) from non-fluorescing (dead). The entire process can be completely quickly. This provides an almost "real-time" assessment of the proportion of a population that has been killed by an antibiotic. If the proportion of dead bacteria is low, resistance is indicated.

All the assessments of antibiotic effectiveness need to be done in a controlled manner. This necessitates the use of standard test types of bacteria (strains that are known to be resistant to the particular antibiotic as well as other strains that are known to be sensitive to the antibiotic). The concentration of the bacteria used is also important. Too many bacteria can "dilute" out the antibiotic, producing a false indication of resistance. Controls need to be included to verify that the experiment was not subject to contamination, otherwise the possibility that a finding of resistance was due to a contaminating bacteria could not be discounted.

In clinical settings, a finding of resistance would prompt the search for another antibiotic. Often, identification of the bacteria will suggest, from previous documented tests of others, an antibiotic to which the organism will be susceptible. But, increasingly, formerly effective antibiotics are losing their potency as bacteria acquire resistance to them. Thus, tests of antibiotic resistance grow in importance.

ANTIBIOTICS

Antibiotics are natural or synthetic compounds that kill **bacteria**. There are a myriad of different antibiotics that act on different structural or biochemical components of bacteria. Antibiotics have no direct effect on virus.

Prior to the discovery of the first antibiotic, **penicillin**, in the 1930s, there were few effective ways of combating bacterial infections. Illnesses such as **pneumonia**, **tuberculosis**, and **typhoid fever** were virtually untreatable, and minor bacterial

infections could blossom into life-threatening maladies. In the decades following the discovery of penicillin, many naturally occurring antibiotics were discovered and still more were synthesized towards specific targets on or in bacteria.

Antibiotics are manufactured by bacteria and various eukaryotic organisms, such as plants, usually to protect the organism from attack by other bacteria. The discovery of these compounds involves screening samples against bacteria for an inhibition in growth of the bacteria. In commercial settings, such screening has been automated so that thousands of samples can be processed each day. Antibiotics can also be manufactured by tailoring a compound to hone in on a selected target. The advent of molecular sequencing technology and three-dimensional image reconstruction has made the design of antibiotics easier.

Penicillin is one of the antibiotics in a class known as beta-lactam antibiotics. This class is named for the ring structure that forms part of the antibiotic molecule. Other classes of antibiotics include the tetracyclines, aminoglycosides, rifamycins, quinolones, and sulphonamides. The action of these antibiotics is varied. For example, beta-lactam antibiotics exert their effect by disrupting the manufacture of **peptidoglycan**, which is main stress-bearing network in the bacterial cell wall. The disruption can occur by blocking either the construction of the subunits of the peptidoglycan or by preventing their incorporation into the existing network. In another example, amonglycoside antibiotics can bind to a subunit of the ribosome, which blocks the manufacture of protein, or can reduce the ability of molecules to move across the cell wall to the inside of the bacterium. As a final example, the quinolone antibiotics disrupt the function of an enzyme that uncoils the double helix of **deoxyribonucleic acid**, which is vital if the **DNA** is to be replicated.

Besides being varied in their targets for antibacterial activity, different antibiotics can also vary in the range of bacteria they affect. Some antibiotics are classified as narrow-spectrum antibiotics. They are lethal against only a few types (or genera) of bacteria. Other antibiotics are active against many bacteria whose construction can be very different. Such antibiotics are described as having a broad-spectrum of activity.

In the decades following the discovery of penicillin, a myriad of different antibiotics proved to be phenomenally effective in controlling infectious bacteria. Antibiotics quickly became (and to a large extent remain) a vital tool in the physician's arsenal against many bacterial infections. Indeed, by the 1970s the success of antibiotics led to the generally held view that bacterial infectious diseases would soon be eliminated. However, the subsequent acquisition of resistance to many antibiotics by bacteria has proved to be very problematic.

Sometimes resistance to an antibiotic can be overcome by modifying the antibiotic slightly, via addition of a different chemical group. This acts to alter the tree-dimensional structure of the antibiotic. Unfortunately, such a modification tends to produce susceptibility to the new antibiotic for a relatively short time.

Ciprofloxacin.

Antibiotic resistance, a problem that develops when antibiotics are overused or misused. If an antibiotic is used properly to treat an infection, then all the infectious bacteria should be killed directly, or weakened such that the host's immune response will kill them. However, the use of too low a concentration of an antibiotic or stopping antibiotic therapy before the prescribed time period can leave surviving bacteria in the population. These surviving bacteria have demonstrated resistance. If the resistance is governed by a genetic alteration, the genetic change may be passed on to subsequent generations of bacterial. For example, many strains of the bacterium that causes tuberculosis are now also resistant to one or more of the antibiotics routinely used to control the lung infection. As a second example, some strains of *Staphylococcus aureus* that can cause boils, pneumonia, or bloodstream infections, are resistant to almost all antibiotics, making those conditions difficult to treat. Ominously, a strain of Staphylococcus (which so far has been rarely encountered) is resistant to all known antibiotics.

See also Bacteria and bacterial infection; Bacterial genetics; *Escherichia coli*; Rare genotype advantage

ANTIBIOTICS, HISTORY OF DEVELOPMENT

• *see* HISTORY OF THE DEVELOPMENT OF ANTIBIOTICS

ANTIBODY-ANTIGEN, BIOCHEMICAL AND MOLECULAR REACTIONS

Antibodies are produced by the **immune system** in response to antigens (material perceived as foreign. The **antibody** response to a particular **antigen** is highly specific and often involves a physical association between the two molecules. This association is governed by biochemical and molecular forces.

In two dimensions, many antibody molecules present a "Y" shape. At the tips of the arms of the molecules are regions that are variable in their amino acid sequences, depending upon the antigen and the antibody formed in response. The

arm-tip regions are typically those that bind to the antigen. These portions of the antibody are also known as the antigenic determinants, or the epitopes.

There are several different types of biochemical interactions between the antibody's epitopes and the target regions on the antigen. Hydrogen bonds are important in stabilizing the antibody-antigen association. In addition, other weak interactions (e.g., van der Waals forces, **hydrophobic** interactions, electrostatic forces) act to tighten the interaction between the regions on the antibody and the antigen.

The hydrogen bonds that are important in antigen-antibody bonding form between amino acids of the antibody and the antigen. Water molecules that fill in the spaces between the antibody and the antigen create other hydrogen bonds. The formation of hydrogen bonds between other regions of the **antibody and antigen**, and the water molecules stabilizes the binding of the immune molecules.

The three-dimensional shape of the molecules is also an important factor in binding between an antibody and an antigen. Frequently, the antibody molecule forms a pocket that is the right size and shape to accommodate the target region of the antigen. This phenomenon was initially described as the "lock and key" hypothesis.

The exact configuration of the antibody-antigen binding site is dependent on the particular antigen. Some antigens have a binding region that is compact. Such a region may be able to fit into a pocket or groove in the antibody molecule. In contrast, other antigen sites may be bulky. In this case, the binding site may be more open or flatter.

These various three dimensional structures for the binding site are created by the sequence of amino acids that comprise the antibody protein. Some sequences are enriched in hydrophobic (water-loving) amino acids. Such regions will tend to form flat sheets, with all the amino acids exposed to the hydrophilic environment. Other sequences of amino acids can contain both hydrophilic and hydrophobic (water-hating) amino acids. The latter will tend to bury themselves away from water via the formation of a helical shape, with the hydrophobic region on the inside. The overall shape of an antibody and antigen depends upon the number of hydrophilic and hydrophobic regions and their arrangement within the protein molecule.

The fact that the interaction between an antibody and an antigen requires a specific three-dimensional configuration is exploited in the design of some vaccines. These vaccines consist of an antibody to a region that is present on a so-called receptor protein. Antigens such as toxin molecules recognize the receptor region and bind to it. However, if the receptor region is already occupied by an antibody, then the binding of the antigen cannot occur, and the deleterious effect associated with binding of the antigen is averted.

Antibody antigen reactions tend to be irreversible under normal conditions. This is mainly due to the establishment of the various chemical bonds and interactions between the molecules. The visible clumping of the antibody-antigen complex seen in solutions and diagnostic tests such as the Ochterlony test is an example of the irreversible nature of the association.

See also Immune system; Immunoglobulins and immunoglobulin deficiency syndromes; Laboratory techniques in immunology; Protein crystallography

ANTIBODY AND ANTIGEN

Antibodies, or Y-shaped **immunoglobulins**, are proteins found in the blood that help to fight against foreign substances called antigens. Antigens, which are usually proteins or polysaccharides, stimulate the **immune system** to produce antibodies. The antibodies inactivate the antigen and help to remove it from the body. While antigens can be the source of infections from pathogenic **bacteria** and **viruses**, organic molecules detrimental to the body from internal or environmental sources also act as antigens. Genetic engineering and the use of various mutational mechanisms allow the construction of a vast array of antibodies (each with a unique genetic sequence).

Specific genes for antibodies direct the construction of antigen specific regions of the antibody molecule. Such antigen-specific regions are located at the extremes of the Y-shaped immunglobulin-molecule.

Once the immune system has created an antibody for an antigen whose attack it has survived, it continues to produce antibodies for subsequent attacks from that antigen. This long-term memory of the immune system provides the basis for the practice of **vaccination** against disease. The immune system, with its production of antibodies, has the ability to recognize, remember, and destroy well over a million different antigens.

There are several types of simple proteins known as **globulins** in the blood: alpha, beta, and gamma. Antibodies are gamma globulins produced by **B lymphocytes** when antigens enter the body. The gamma globulins are referred to as immunoglobulins. In medical literature they appear in the abbreviated form as Ig. Each antigen stimulates the production of a specific antibody (Ig).

Antibodies are all in a Y-shape with differences in the upper branch of the Y. These structural differences of amino acids in each of the antibodies enable the individual antibody to recognize an antigen. An antigen has on its surface a combining site that the antibody recognizes from the combining sites on the arms of its Y-shaped structure. In response to the antigen that has called it forth, the antibody wraps its two combining sites like a "lock" around the "key" of the antigen combining sites to destroy it.

An antibody's mode of action varies with different types of antigens. With its two-armed Y-shaped structure, the antibody can attack two antigens at the same time with each arm. If the antigen is a toxin produced by pathogenic bacteria that cause an infection like **diphtheria** or **tetanus**, the binding process of the antibody will nullify the antigen's toxin. When an antibody surrounds a virus, such as one that causes **influenza**, it prevents it from entering other body cells. Another mode of action by the antibodies is to call forth the assistance of a group of immune agents that operate in what is known as the plasma **complement** system. First, the antibodies will coat infectious bacteria and then white blood cells will

complete the job by engulfing the bacteria, destroying them, and then removing them from the body.

There are five different antibody types, each one having a different Y-shaped configuration and function. They are the Ig G, A, M, D, and E antibodies.

IgG is the most common type of antibody. It is the chief Ig against microbes. It acts by coating the microbe to hasten its removal by other immune system cells. It gives lifetime or long-standing **immunity** against infectious diseases. It is highly mobile, passing out of the blood stream and between cells, going from organs to the skin where it neutralizes surface bacteria and other invading **microorganisms**. This mobility allows the antibody to pass through the placenta of the mother to her fetus, thus conferring a temporary defense to the unborn child.

After birth, IgG is passed along to the child through the mother's milk, assuming that she nurses the baby. But some of the Ig will still be retained in the baby from the placental transmission until it has time to develop its own antibodies. Placental transfer of antibodies does not occur in horses, pigs, cows, and sheep. They pass their antibodies to their offspring only through their milk.

This antibody is found in body fluids such as tears, saliva, and other bodily secretions. It is an antibody that provides a first line of defense against invading pathogens and allergens, and is the body's major defense against viruses. It is found in large quantities in the bloodstream and protects other wet surfaces of the body. While they have basic similarities, each IgA is further differentiated to deal with the specific types of invaders that are present at different openings of the body.

Since this is the largest of the antibodies, it is effective against larger microorganisms. Because of its large size (it combines five Y-shaped units), it remains in the bloodstream where it provides an early and diffuse protection against invading antigens, while the more specific and effective IgG antibodies are being produced by the plasma cells.

The ratio of IgM and IgG cells can indicate the various stages of a disease. In an early stage of a disease there are more IgM antibodies. The presence of a greater number of IgG antibodies would indicate a later stage of the disease. IgM antibodies usually form clusters that are in the shape of a star.

This antibody appears to act in conjunction with B and T-cells to help them in location of antigens. Research continues on establishing more precise functions of this antibody.

The antibody responsible for allergic reactions, IgE acts by attaching to cells in the skin called mast cells and basophil cells (mast cells that circulate in the body). In the presence of environmental antigens like pollens, foods, chemicals, and drugs, IgE releases histamines from the mast cells. The histamines cause the nasal **inflammation** (swollen tissues, running nose, sneezing) and the other discomforts of hay fever or other types of allergic responses, such as hives, asthma, and in rare cases, anaphylactic shock (a life-threatening condition brought on by an allergy to a drug or insect bite). An explanation for the role of IgE in allergy is that it was an antibody that was useful to early man to prepare the immune system to fight **parasites**. This function is presently overextended in reacting to environmental antigens.

The presence of antibodies can be detected whenever antigens such as bacteria or red blood cells are found to agglutinate (clump together), or where they precipitate out of solution, or where there has been a stimulation of the plasma complement system. Antibodies are also used in laboratory tests for blood typing when transfusions are needed and in a number of different types of clinical tests, such as the Wassermann test for **syphilis** and tests for **typhoid fever** and infectious **mononucleosis**.

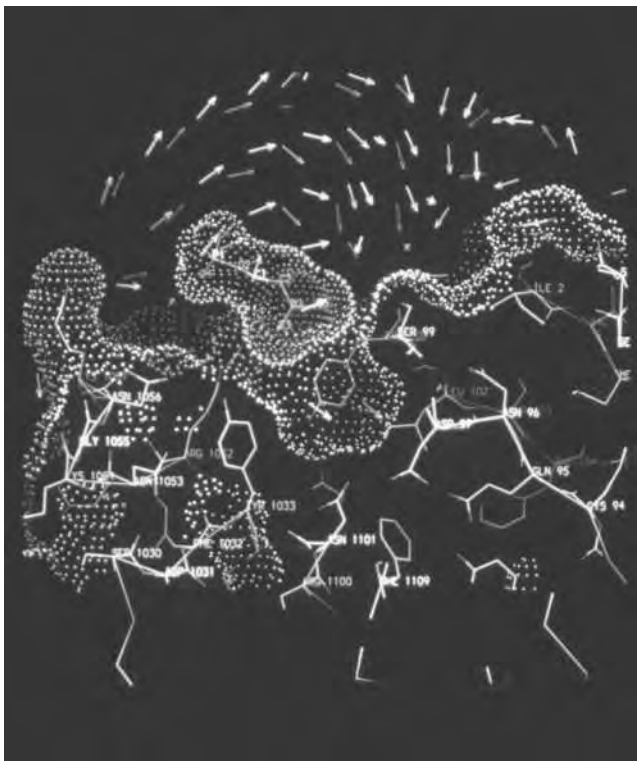
By definition, anything that makes the immune system respond to produce antibodies is an antigen. Antigens are living foreign bodies such as viruses, bacteria, and **fungi** that cause disease and infection. Or they can be dust, chemicals, pollen grains, or food proteins that cause allergic reactions.

Antigens that cause allergic reactions are called allergens. A large percentage of any population, in varying degrees, is allergic to animals, fabrics, drugs, foods, and products for the home and industry. Not all antigens are foreign bodies. They may be produced in the body itself. For example, cancer cells are antigens that the body produces. In an attempt to differentiate its "self" from foreign substances, the immune system will reject an organ transplant that is trying to maintain the body or a blood transfusion that is not of the same blood type as itself.

There are some substances such as nylon, plastic, or Teflon that rarely display antigenic properties. For that reason, nonantigenic substances are used for artificial blood vessels, component parts in heart pacemakers, and needles for hypodermic syringes. These substances seldom trigger an immune system response, but there are other substances that are highly antigenic and will almost certainly cause an immune system reaction. Practically everyone reacts to certain chemicals, for example, the resin from the poison ivy plant, the venoms from insect and reptile bites, solvents, formalin, and asbestos. Viral and bacterial infections also generally trigger an antibody response from the immune system. For most people **penicillin** is not antigenic, but for some there can be an immunological response that ranges from severe skin rashes to death.

Another type of antigen is found in the tissue cells of organ transplants. If, for example, a kidney is transplanted, the surface cells of the kidney contain antigens that the new host body will begin to reject. These are called **human leukocyte antigens** (**HLA**), and there are four major types of HLA subdivided into further groups. In order to avoid organ rejection, tissue samples are taken to see how well the new organ tissues match for HLA compatibility with the recipient's body. Drugs will also be used to suppress and control the production of helper/suppressor T-cells and the amount of antibodies.

Red blood cells with the ABO antigens pose a problem when the need for blood transfusions arises. Before a transfusion, the blood is tested for type so that a compatible type is used. Type A blood has one kind of antigen and type B another. A person with type AB blood has both the A and B antigen. Type O blood has no antigens. A person with type A blood would require either type A or O for a successful transfusion. Type B and AB would be rejected. Type B blood would



Binding of an antibody with an antigen, as detected using X-ray crystallography.

be compatible with a B donor or an O donor. Since O has no antigens, it is considered to be the universal donor. Type AB is the universal recipient because its antibodies can accept A, B, AB, or O. One way of getting around the problem of blood types in transfusion came about as a result of World War II. The great need for blood transfusions led to the development of blood plasma, blood in which the red and white cells are removed. Without the red blood cells, blood could be quickly administered to a wounded soldier without the delay of checking for the blood antigen type.

Another antigenic blood condition can affect the life of newborn babies. Rhesus disease (also called erythroblastosis fetalis) is a blood disease caused by the incompatibility of **Rh** factors between a fetus and a mother's red blood cells. When an Rh negative mother gives birth to an Rh positive baby, any transfer of the baby's blood to the mother will result in the production of antibodies against Rh positive red blood cells. At her next pregnancy the mother will then pass those antibodies against Rh positive blood to the fetus. If this fetus is Rh positive, it will suffer from Rh disease. Tests for Rh blood factors are routinely administered during pregnancy.

Western medicine's interest in the practice of vaccination began in the eighteenth century. This practice probably originated with the ancient Chinese and was adopted by Turkish doctors. A British aristocrat, Lady Mary Wortley Montagu (1689–1762), discovered a crude form of vaccination taking place in a lower-class section of the city of Constantinople while she was traveling through Turkey. She described her experience in a letter to a friend. Children who were injected with pus from a **smallpox** victim did not die from the disease but built up immunity to it. Rejected in England by most doctors who thought the practice was barbarous, smallpox vaccination was adopted by a few English physicians of the period. They demonstrated a high rate of effectiveness in smallpox prevention.

By the end of the eighteenth century, **Edward Jenner** (1749–1823) improved the effectiveness of vaccination by injecting a subject with **cowpox**, then later injecting the same subject with smallpox. The experiment showed that immunity against a disease could be achieved by using a **vaccine** that did not contain the specific pathogen for the disease. In the nineteenth century, **Louis Pasteur** (1822–1895) proposed the **germ theory of disease**. He went on to develop a **rabies** vaccine that was made from the spinal cords of rabid rabbits. Through a series of injections starting from the weakest strain of the disease, Pasteur was able, after 13 injections, to prevent the death of a child who had been bitten by a rabid dog.

There is now greater understanding of the principles of vaccines and the immunizations they bring because of our knowledge of the role played by antibodies and antigens within the immune system. Vaccination provides active immunity because our immune systems have had the time to recognize the invading germ and then to begin production of specific antibodies for the germ. The immune system can continue producing new antibodies whenever the body is attacked again by the same organism or resistance can be bolstered by booster shots of the vaccine.

For research purposes there were repeated efforts to obtain a laboratory specimen of one single antibody in sufficient quantities to further study the mechanisms and applications of antibody production. Success came in 1975 when two British biologists, **César Milstein** (1927–) and Georges Kohler (1946–) were able to clone immunoglobulin (Ig) cells of a particular type that came from multiple myeloma cells. Multiple myeloma is a rare form of cancer in which white blood cells keep turning out a specific type of Ig antibody at the expense of others, thus making the individual more susceptible to outside infection. By combining the myeloma cell with any selected antibody-producing cell, large numbers of specific monoclonal antibodies can be produced. Researchers have used other animals, such as mice, to produce hybrid antibodies which increase the range of known antibodies.

Monoclonal antibodies are used as drug delivery vehicles in the treatment of specific diseases, and they also act as catalytic agents for protein reactions in various sites of the body. They are also used for diagnosis of different types of diseases and for complex analysis of a wide range of biological substances. There is hope that monoclonal antibodies will be as effective as **enzymes** in chemical and technological processes, and that they currently play a significant role in genetic engineering research.

See also Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Antibody, mono-

clonal; Antigenic mimicry; Immune stimulation, as a vaccine; Immunologic therapies; Infection and resistance; Infection control; Major histocompatibility complex (MHC)

ANTIBODY FORMATION AND KINETICS

Antibody formation occurs in response to the presence of a substance perceived by the **immune system** as foreign. The foreign entity is generically called an **antigen**. There are a myriad of different antigens that are presented to the immune system. Hence, there are a myriad of antibodies that are formed.

The formation of innumerable antibodies follows the same general pattern. First, the immune system discriminates between host and non-host antigens and reacts only against those not from the host. However, malfunctions occur. An example is rheumatoid arthritis, in which a host response against self-antigens causes the deterioration of bone. Another example is heart disease caused by a host reaction to a heart muscle protein. The immune response is intended for an antigen of a bacterium called Chlamydia, which possess an antigen very similar in structure to the host heart muscle protein.

Another feature of antibody formation is that the production of an antibody can occur even when the host has not "seen" the particular antigen for a long time. In other words, the immune system has a memory for the antigenic response. Finally, the formation of an antibody is a very precise reaction. Alteration of the structure of a protein only slightly can elicit the formation of a different antibody.

The formation of antibody depends upon the processing of the incoming antigen. The processing has three phases. The first phase is the equilibration of an antigen between the inside and outside of cells. Soluble antigens that can dissolve across the cell membranes are able to equilibrate, but more bulky antigens that do not go into solution cannot. The second phase of antigen processing is known as the catabolic decay phase. Here, cells such as macrophages take up the antigen. It is during this phase that the antigen is "presented" to the immune system and the formation of antibody occurs. The final phase of antigen processing is called the immune elimination phase. The coupling between antigen and corresponding antibody occurs, and the complex is degraded. The excess antibody is free to circulate in the bloodstream.

The antibody-producing cell of the immune system is called the lymphocyte or the B cell. The presentation of a protein target stimulates the lymphocyte to divide. This is termed the inductive or lag phase of the primary antibody response. Some of the daughter cells will then produce antibody to the protein target. With time, there will be many daughter lymphocytes and much antibody circulating in the body. During this log or exponential phase, the quantity of antibody increases rapidly.

For a while, the synthesis of antibody is balanced by the breakdown of the antibody, so the concentration of antibody stays the same. This is the plateau or the steady-state phase. Within days or weeks, the production of the antibody slows. After this decline or death phase, a low, baseline concentration may be maintained.

The lymphocytes retain the memory of the target protein. If the antigen target appears, as happens in the second **vaccination** in a series, the pre-existing, "primed" lymphocytes are stimulated to divide into antibody-producing daughter cells. Thus, the second time around, a great deal more antibody is produced. This primed surge in antibody concentration is the secondary or anamnestic (memory) response. The higher concentration of antibody can be maintained for months, years, or a lifetime.

Another aspect of antibody formation is the change in the class of antibodies that are produced. In the primary response, mainly the IgM class of antibody is made. In the secondary response, IgG, IgE, or IgA types of antibodies are made.

The specificity of an antibody response, while always fairly specific, becomes highly specific in a secondary response. While in a primary response, an antibody may cross-react with antigens similar to the one it was produced in response to, such cross-reaction happens only very rarely in a secondary response. The binding between **antibody and antigen** becomes tighter in a secondary response as well.

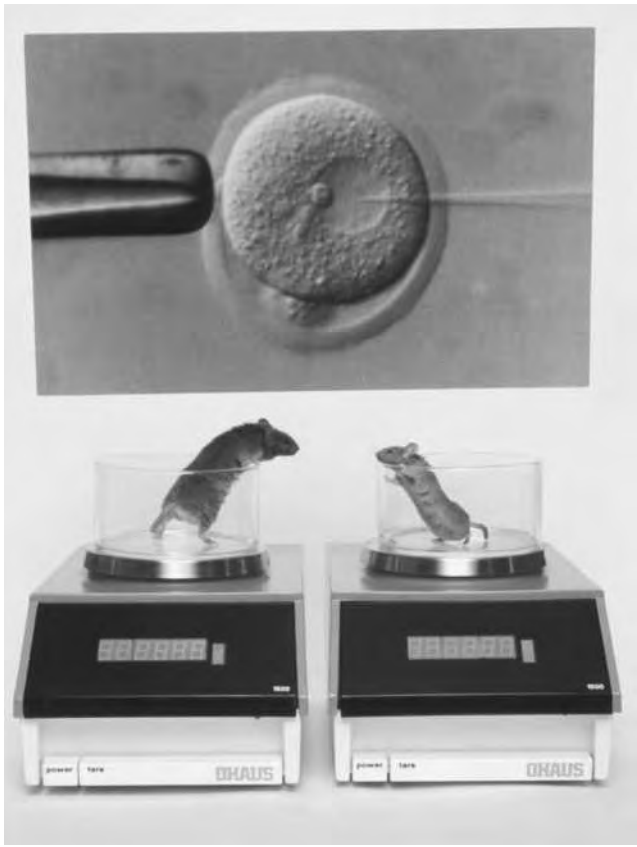
See also Antigenic mimicry; History of immunology; Immunoglobulins and immunoglobulin deficiency syndromes; Laboratory techniques in immunology; Streptococcal antibody tests

ANTIBODY, MONOCLONAL

The **immune system** of vertebrates help keep the animal healthy by making millions of different proteins (**immunoglobulins**) called antibodies to disable antigens (harmful foreign substances such as toxins or **bacteria**). Scientists have worked to develop a method to extract large amounts of specific antibodies from clones (exact copies) of a cell created by fusing two different natural cells. Those antibodies are called monoclonal antibodies.

Antibody research began in the 1930s when the American pathologist **Karl Landsteiner** found that animal antibodies counteract specific antigens and that all antibodies have similar structures. Research by the American biochemists Rodney R. Porter (1917–1985) and **Gerald M. Edelman** (1929–) during the 1950s determined antibody structure, and particularly the active areas of individual antibodies. For their work they received the 1972 Nobel prize in physiology or medicine.

By the 1960s, scientists who studied cells needed large amounts of specific antibodies for their research, but several problems prevented them from obtaining these antibodies. Animals can be injected with antigens so they will produce the desired antibodies, but it is difficult to extract them from among the many types produced. Attempts to reproduce various antibodies in an artificial environment encountered some complications. Lymphocytes, the type of cell that produces specific antibodies, are very difficult to grow in the laboratory; conversely, tumor cells reproduce easily and endlessly, but make only their own types of antibodies. A bone marrow tumor called a myeloma interested scientists because it begins



Mice used to develop the monoclonal cells that secret a specific antibody.

from a single cell that produces a single antibody, then divides many times. The cells that divided do not contain antibodies and could, therefore, be crossed with lymphocytes to produce specific antibodies. These hybrid cells are called hybridoma, and they produce monoclonal antibodies.

One molecular biologist who needed pure antibodies for a study of myeloma **mutations** was the Argentinean **César Milstein** (1927–). After receiving a doctorate in **biochemistry**, specializing in **enzymes**, from the University of Buenos Aires in 1957, he continued this study at the University of Cambridge in England. There he worked under the biochemist Frederick Sanger and earned another doctorate in 1961. Milstein had returned to Argentina, but political disturbances forced him to flee the country. He came back to Cambridge, where Sanger suggested that he work with antibodies.

In 1974, Milstein was working with **Georges Köhler** (1946–1995), a German postdoctoral student who had just received his doctorate from the University of Freiburg for work performed at the Institute for **Immunology** in Basel, Switzerland. To produce the needed antibodies, Milstein and Köhler first injected a mouse with a known **antigen**. After extracting the resulting lymphocytes from the mouse's blood, they fused one of them with a myeloma cell. The resulting hybrid produced the lymphocyte's specific antibody and reproduced endlessly. As Milstein soon realized, their technique for producing monoclonal antibodies could be used in many capacities. Milstein and Köhler shared part of the 1984 Nobel prize in physiology or medicine for their invention.

Today pure antibodies are made using the Milstein-Köhler technique and also through genetic engineering, which adds the **gene** for the desired antibody to bacteria that can produce it in large amounts. Monoclonal antibodies are instrumental in the performance of sensitive medical diagnostic tests such as: determining pregnancy with chorionic gonadotropin; determining the amino acid content of substances; classifying antigens; purifying hormones; and modifying infectious or toxic substances in the body. They are also important in cancer treatment because they can be tagged with radioisotopes to make images of tumors.

See also Antibody-antigen, biochemical and molecular reactions; Antibody and antigen; Immunity, cell mediated; Immunogenetics; Immunologic therapies; Immunological analysis techniques; *In vitro* and *in vivo* research

ANTIGEN • *see* ANTIBODY AND ANTIGEN

ANTIGENIC MIMICRY

Antigenic mimicry is the sharing of antigenic sites between **microorganisms** and mammalian tissue. An immune response can be directed both at the microorganism and at the host site that shares the antigenic determinant. This autoimmune response due to antigenic mimicry is known to be a crucial factor in the development of certain ailments in humans.

The **immune system** recognizes three-dimensional structure of protein. A protein, which is made up of a sequence of amino acids strung together, will fold up in various ways, depending on whether a section is more hydrophilic ("water loving") or **hydrophobic** ("water hating"), and depending on the function of various regions of the protein.

Proteins that adopt a similar three-dimensional configuration can stimulate a common response from the immune system. Typically, proteins that have a similar amino acid sequence will adopt the similar folded structures. For example, the **bacteria** *Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Chlamydia trachomatis* possess a protein that is part of the bacterial outer membrane. This protein is similar in amino acid sequence to a portion of a protein, called alpha-myosin heavy chain, which is found specifically in the heart muscle of humans and animals such as mice. In mice, an immune reaction to Chlamydia triggers a condition known as inflammatory heart disease. A continued host autoimmune response damages the heart, leading to cardiac malfunction. Indeed, it has been shown that a significant number of patients with heart disease have antibodies to Chlamydia in their blood, indicative of a past infection with the bacteria.

Rheumatoid arthritis is another example of a malady that is the consequence of an autoimmune reaction.

The Chlamydia studies have pointed out the widespread nature of antigenic mimicry. Other bacteria, **viruses**, **fungi** and **protozoa** share the antigenic similarity with the mouse antigenic region. The bacteria include *Borrelia burgdorferi* (the agent of **Lyme disease**), *Treponema pallidum* (the causative agent of **syphilis**), and *Mycoplasma pneumoniae* (the cause of non-viral atypical **pneumonia**).

Antigenic mimicry may also be the basis of the ulcers formed upon infection of humans with *Helicobacter pylori*. The acidic environment of the stomach would exacerbate host tissue damage due to an autoimmune response.

Antigenic mimicry supports a hypothesis known as the "infection hypothesis," which proposes that common human diseases are caused by infections. If so, then treatment for heart disease and stomach ulcers would involve strategies to eliminate bacterial infections.

See also Bacteria and bacterial infection; Immunit: active, passive, and delayed

ANTISEPTICS

Antiseptics are compounds that act to counteract sepsis, which is an illness caused by a **bacterial infection** of the blood. Antiseptics are able to counteract sepsis by preventing the growth of pathogenic (disease causing) **microorganisms**. An antiseptic may kill a microorganism, but it does not necessarily have to. The treated microbes may only be weakened. The weaker, slower growing microbes may then be more susceptible to the defense mechanisms of the host.

The terms antiseptic and disinfectant are used almost interchangeably nowadays. Yet they do have different meanings. An antiseptic is a chemical or technique that is used on people. A disinfectant is a chemical that is applied to an inanimate object or surface to get rid of microorganisms. An antiseptic generally does not have the same potency as a disinfectant. Otherwise, the chemical would harm the tissues it is in contact with. For this reason, an antiseptic should not be used to treat inanimate objects. Likewise, the generally more toxic disinfectant should not be used to treat skin or areas such as the mucous membranes of the nose.

While more is known of the molecular basis of antiseptic actions, the use of antimicrobial compounds is ancient. For example, the black eye make-up known as kohl, which was used by the ancient Arabs and Egyptians, is a mixture of copper and antimony. These compounds are antiseptic. Indeed, the modern cure for trachoma (blindness caused by infection of the eyes by the bacterium *Chlamydia trachomatis*) is remarkably similar in composition to kohl.

There are a number of antiseptics and antiseptic procedures.

In a health care setting, powerful antiseptics are used to ensure that the skin is essentially sterile prior to an operation. Examples of such antiseptics include chlorhexidine and iodophors (iodine-containing compounds). Alcohol is an antiseptic, which is routinely used to swab the skin prior to an injection. Alcohol acts to coagulate the protein in **bacteria**. The irreversible change in the protein is lethal to the bacteria. In the example of the injection, alcohol swabbing of the injection site will kill the bacteria on the skin, so that living bacteria are not carried into the body upon insertion of the needle. Dilution of alcohol, so that a solution is 30% alcohol by volume, makes this antiseptic even more potent, as it allows the alcohol to permeate into the bacteria. Pure alcohol rapidly coagulates surface proteins, producing a coagulated crust around the bacteria.

Another antiseptic is carbolic acid. This is also known as phenol. The coal tar-based product was discovered in 1834. Originally phenol was poured down sewers to kill microorganisms. Over time, its use expanded. In 1863, the British surgeon **Joseph Lister** began using a spray of phenol to disinfect open wounds during surgery. Prior to his innovation, such surgery was only performed when all other avenues of treatment had failed, since the risk of death from infection was extremely high.

Still another antiseptic compound is pine oil. It is added to household disinfectants more because of its pleasant smell than its aseptic power nowadays. In fact, it inclusion actually weakens the bacteria-killing power of the household disinfectant.

Lister's method was supplanted by the adoption of extreme cleanliness in the operating room, such as the use of sterile masks, gloves and gowns, in order to keep the surgical area free of microorganisms. This approach is known as antiseptic surgery. As strange as it may seem now, surgeons in Lister's era often did not change or clean their operating garb between operations. A surgeon would often commence an operation wearing a gown covered with the blood and germs of many previous operations. Prior to the introduction of antisepsis in the operating room, the rate of death following surgery was almost 60%. After the introduction of antisepsis, the recorded death rate in England dropped to four per cent.

Hand washing has also become standard practice in the hospital and the home.

Another antiseptic technique is **sterilization**. The use of steam at higher than atmospheric pressure is an effective means of killing many types of bacteria, including those that form spores.

In the home, antiseptics are often evident as lotions or solutions that are applied to a cut or scrape to prevent infection. For these uses, it is necessary to clean the affected area of skin first to dislodge any dirt or other material that could reduce the effectiveness of the antiseptic. Antiseptics, particularly those used in the home, are designed for a short-term use to temporarily rid the skin of microbes. The skin, being in primary contact with the environment, will quickly become recolonized with microorganisms. Long-term use of antiseptics encourages the development of populations of microorganisms that are resistant to the antiseptic. Additionally, the skin can become irritated by the long exposure to the harsh chemical. Some people can even develop **allergies** to the antiseptic.

Another hazard of antiseptics that has only become apparent since the 1990s is the **contamination** of the environment. Antiseptic solutions that are disposed of in sinks and toi-

lets can make their way to rivers and lakes. Contamination of the aquifer (the surface or underground reserve of water from which drinking water is obtained) has become a real possibility.

See also Antibiotics; Infection control

ANTISERUM AND ANTITOXIN

Both antisera and antitoxins are means of proactively combating infections. The introduction of compounds to which the **immune system** responds is an attempt to build up protection against **microorganisms** or their toxins before the microbes actually invade the body.

The use of antiserum and antitoxin preparations is now a standard avenue of **infection control**. The beginnings of the strategies dates to the time of **Edward Jenner** in the late eighteenth century. Then, Jenner used an inoculum of **cowpox** material to elicit protection against the **smallpox** virus.

Jenner's strategy of using a live organism to elicit an **antibody** response led to a "third-party" strategy, whereby serum is obtained from an animal that has been exposed to an **antigen** or to the microorganism that contains the antigen. This so-called antiserum is injected into the human to introduce the protective antibodies directly, rather than having them manufactured by the person's own immune system.

The same strategy produces antitoxin. In this case, the material injected into the animal would consist of active toxin, but in very low quantities. The intent of the latter is to stimulate antibody production against a toxin that has not been changed by the procedures used to inactivate toxin activity.

The use of antitoxin has been largely supplanted by the injection of a crippled form of the toxin of interest (also known as a toxoid) or a particularly vital fragment of the toxin that is needed for toxic activity. The risk of the use of a toxoid or a fragment of toxin is that the antibody that is produced is sufficiently different from that produced against the real target so as to be ineffective in a person.

Since the time of Jenner, a myriad of antisera and antitoxins have been produced against bacterial, viral and protozoan diseases. The results of their use can be dramatic. For example, even in the 1930s, the form of **influenza** caused by the bacterium *Hemophilus influenzae* was almost always lethal to infants and children. Then, Elizabeth Hattie, a pediatrician and microbiologist, introduced an anti-influenzal antiserum produced in rabbits. The use of this antiserum reduced *Hemophilus influenzae* influenza-related mortality to less than twenty per cent.

Antiserum can contain just one type of antibody, which is targeted at a single antigen. This is known as monovalent antiserum. Or, the antiserum can contain multiple antibodies, which are directed at different antibody targets. This is known as polyvalent antiserum.

The indirect protective effect of antiserum and antitoxin is passive **immunity**. That is, a protective response is produced in someone who has not been immunized by direct exposure to the organism. Passive immunity provides immediate but temporary protection.

Antiserum and antitoxin are obtained from the blood of the test animal. The blood is obtained at a pre-determined time following the injection of the antigen, microorganism, or toxoid. The antiserum constitutes part of the plasma, the clear component of the blood that is obtained when the heavier blood cells are separated by spinning the blood in a machine called a centrifuge.

Examples of antisera are those against **tetanus** and **rabies**. Typically, these antisera are administered if someone has been exposed to an environment or, in the case of rabies, an animal, which makes the threat of acquiring the disease real. The antisera can boost the chances of successfully combating the infectious organism. After the threat of disease is gone, the protective effect is no longer required.

The advent of **antibiotics** has largely replaced some types of antiserum. This has been a positive development, for antiserum can cause allergic reactions that in some people are fatal. The allergic nature of antiserum, which is also known as serum shock, arises from the nature of its origin. Because it is derived from an animal, there may be components of the animal present in the antiserum. When introduced into a human, the animal proteins are themselves foreign, and so will produce an immune response. For this reason antiserum is used cautiously today, as in the above examples. The risk of the use of antiserum or antitoxin is more than compensated for by the risk of acquiring a life-threatening malady if treatment is not undertaken.

Serum sickness is a hypersensitive immune reaction to a contaminating animal protein in the antiserum. The antibodies that are produced bind to the antigen to make larger particles called immune complexes. The complexes can become deposited in various tissues, causing a variety of symptoms. The symptoms typically do not appear for a few weeks after the antiserum or antitoxin has been administered.

With the development of sophisticated techniques to examine the genetic material of microorganisms and identify genes that are responsible for the aspects of disease, the use of antiserum and antitoxin may enter a new phase of use. For example, the genetic sequences that are responsible for the protein toxins of the **anthrax** bacterium are now known. From these sequences the proteins they encode can be manufactured in pure quantities. These pure proteins can then form the basis of an antitoxin. The antibodies produced in animals can be obtained in very pure form as well, free of contaminating animal proteins. These antibodies will block the binding of the toxin to host tissue, which blocks the toxic effect. In this and other cases, such as an antitoxin being developed to *Escherichia coli O157:H7*, the use of antitoxin is superior strategy to the use of antibiotics. Antibiotics are capable of killing the anthrax bacterium. They have no effect, however, on action of the toxin that is released by the **bacteria**.

See also Anti-adhesion methods; Antiviral drugs; *E. coli* 0157:H7 infection; *Escharichia coli*; Immune stimulation, as a vaccine; Immunization

ANTIVIRAL DRUGS

Antiviral drugs are compounds that are used to prevent or treat viral infections, via the disruption of an infectious mechanism used by the virus, or to treat the symptoms of an infection.

Different types of antiviral drugs have different modes of operation. For example, acyclovir is a drug that is used to treat the symptoms of the infections arising from the herpes virus family. Such infection includes lesions on the genitals, oral region, or in the brain. Acyclovir is also an antiviral agent in the treatment of chickenpox in children and adults, and shingles in adults caused by the reactivation of the chickenpox virus after a period of latency. Shingles symptoms can also be treated by the administration of valacyclovir and famciclovir.

Eye infections caused by cytomegalovirus can be treated with the antiviral agent known as ganciclovir. The drug acts to lessen the further development and discomfort of the eye irritation. But, the drug may be used as a preventative agent in those people whose **immune system** will be compromised by the use of an immunosupressant.

Another category of antiviral drugs is known as the antiretroviral drugs. These drugs target those **viruses** of clinical significance called **retroviruses** that use the mechanism of reverse **transcription** to manufacture the genetic material needed for their replication. The prime example of a retrovirus is the **Human immunodeficiency virus** (**HIV**), which is the viral agent of acquired **immunodeficiency** syndrome (**AIDS**). The development of antiviral drugs has been stimulated by the efforts to combat HIV. Some anti-HIV drugs have shown promise against **hepatitis** B virus, **herpes** simplex virus, and varicella-zoster virus.

The various antiviral agents are designed to thwart the replication of whatever virus they are directed against. One means to achieve this is by blocking the virus from commandeering the host cell's nuclear replication machinery in order to have its genetic material replicated along with the host's genetic material. The virus is not killed directly. But the prevention of replication will prevent the numbers of viruses from increasing, giving the host's immune system time to deal with the stranded viruses.

The incorporation of the nucleotide building blocks into **deoxyribonucleic acid** (**DNA**) can be blocked using the drug idoxuridine or trifluridine. Both drugs replace the nucleoside thymidine, and its incorporation produces a nonfunctional DNA. However, the same thing happens to the host DNA. So, this antiviral drug is also an anti-host drug. Vidarabine is another drug that acts in a similar fashion. The drug is incorporated into DNA in place of adenine. Other drugs that mimic other DNA building blocks.

Blockage of the viral replicative pathway by mimicking nucleosides can be successful. But, because the virus utilizes the host's genetic machinery, stopping the viral replication usually affects the host cell.

Another tact for antiviral drugs is to block a viral enzyme whose activity is crucial for replication of the viral genetic material. This approach has been successfully exploited by the drug acyclovir. The drug is converted in the host cell to a compound that can out compete another compound for the binding of the viral enzyme, DNA polymerase, which is responsible for building DNA. The incorporation of the acyclovir derivative exclusively into the viral DNA stops the formation of the DNA. Acyclovir has success against herpes simplex viruses, and **Epstein-Barr virus**. Another drug that acts in a similar fashion is famiciclovir.

Other antiviral drugs are directed at the **translation** process, whereby the information from the viral genome that has been made into a template is read to produce the protein product. For example, the drug ribavirin inhibits the formation of messenger **ribonucleic acid**.

Still other antiviral drugs are directed at earlier steps in the viral replication pathway. Amantadine and rimantadine block the **influenza** A virus from penetrating into the host cell and releasing the nuclear material.

Antiviral therapy also includes molecular approaches. The best example is the use of oligonucleotides. These are sequences of nucleotides that are specifically synthesized to be complimentary with a target sequence of viral ribonucleic acid. By binding to the viral **RNA**, the oligonucleotide blocks the RNA from being used as a template to manufacture protein.

The use of antiviral drugs is not without risk. Host cell damage and other adverse host reactions can occur. Thus, the use of antiviral drugs is routinely accompanied by close clinical observation.

See also Immunodeficiency diseases; Viruses and responses to viral infection

APPERT, NICOLAS FRANÇOIS (1750-1841)

French chef

Nicolas Appert gave rise to the food canning industry. Born in Châlons-sur-Marne, France, around 1750, young Appert worked at his father's inn and for a noble family as a chef and wine steward. By 1780 he had set up a confectionery shop in Paris, France.

Appert became interested in **food preservation** when the French government offered a 12,000-franc prize in 1795 to the person who could find a way to keep provisions for Napoleon's armies from spoiling in transit and storage. After years of experimentation Appert devised a method of putting food in glass bottles that were then loosely corked and immersed in boiling water for lengths of time that varied with the particular food; after boiling, the corks were sealed down tightly with wire. In an age before bacteriology, Appert did not comprehend the fact that the heat destroyed **microorganisms** in the food, but he could see that his method—which became known as appertization—preserved the food. Appert later set up his first bottling plant at Massy, south of Paris, in 1804.

The French navy successfully used Appert's products in 1807, and in 1809 Appert was awarded the 12,000-franc prize. A condition of the award was that Appert make public his discovery, which he did in his 1810 work *The Art of Preserving Animal and Vegetable Substances for Several Years,* which gave specific directions for canning over fifty different foods.

This volume spread knowledge about canning around the world and launched what would become a vast industry.

In 1812 Appert used his prize money to make his Massy plant into the world's first commercial cannery, which remained in operation until 1933. Appert, who also invented the bouillon cube, was financially ruined in 1814 when his plant was destroyed during the Napoleonic wars. He died in poverty in 1841.

See also Food preservation; Food safety

ARCHAEA

Genes that code for vital cellular functions are highly conserved through evolutionary time, and because even these genes experience random changes over time, the comparison of such genes allows the relatedness of different organisms to be assessed. American microbiologist Carl Woese and his colleagues obtained sequences of the genes coding for **RNA** in the subunit of the ribosome from different organisms to argue that life on Earth is comprised of three primary groups, or domains. These domains are the Eukarya (which include humans), **Bacteria**, and Archaea.

While Archae are **microorganisms**, they are no more related to bacteria than to **eukaryotes**. They share some traits with bacteria, such as having a single, circular molecule of **DNA**, the presence of more mobile pieces of genetic material called **plasmids**, similar **enzymes** for producing copies of DNA. However, their method of protein production and organization of their genetic material bears more similarity to eukaryotic cells.

The three domains are thought to have diverged from one another from an extinct or as yet undiscovered ancestral line. The archae and eukarya may have branched off from a common ancestral line more recently than the divergence of these two groups from bacteria. However, this view remains controversial and provisional.

The domain Archae includes a relatively small number of microoganisms. They inhabit environments which are too harsh for other microbes. Such environments include hot, molten vents at the bottom of the ocean, the highly salt water of the Great Salt Lake and the Dead Sea, and in the hot sulfurous springs of Yellowstone National Park. Very recently, it has been shown that two specific archael groups, pelagic euryarchaeota and pelagic crenarchaeota are one of the ocean's dominant cell types. Their dominance suggests that they have a fundamentally important function in that ecosystem.

See also Bacterial kingdoms; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses

ARENAVIRUS

Arenavirus is a virus that belongs in a viral family known as Arenaviridae. The name arenavirus derives from the appearance of the spherical virus particles when cut into thin sections and viewed using the transmission **electron microscope**. The interior of the particles is grainy or sandy in appearance, due to the presence of **ribosomes** that have been acquired from the host cell. The Latin designation "arena" means "sandy."

Arenaviruses contain **ribonucleic acid** (**RNA**) as their genetic material. The viral genome consists of two strands of RNA, which are designated the L and S RNA. The ribosomes of the host that are typically present inside the virus particle are used in the manufacture of the components that will be assembled to produce the new virus particles. Little is known about the actual replication of new viral components or about the assembly of these components to produce the new virus particles. It is known that the new virus exits the host by "budding" off from the surface of the host cell. When the budding occurs some of the lipid constituent from the membrane of the host forms the envelope that surrounds the virus.

Those arenaviruses that are of concern to human health are typically transmitted to humans from rats and mice. The only known exception is an arenavirus called the Tacaribe virus, which is resident in *Artibeus* bats. The association between an arenavirus type and a particular species of rodent is specific. Thus, a certain arenavirus will associate with only one species of mouse or rat. There are 15 arenaviruses that are known to infect animals. A hallmark of arenaviruses is that the infections in these rodent hosts tend not to adversely affect the rodent.

Of the fifteen **viruses** that are resident in the animals, five of these viruses are capable of being transmitted to humans. When transmitted to humans, these arenaviruses can cause illness. In contrast to the rodent hosts, the human illness can be compromising.

Most arenavirus infections produce relatively mild symptoms that are reminiscent of the flu, or produce no symptoms whatsoever. For example an arenavirus designated lymphocytic choriomeningitis virus, usually produces symptoms that are mild and are often mistaken for gastrointestinal upset. However, some infections with the same virus produce a severe illness that characterized by an **inflammation** of the sheath that surrounds nerve cells (**meningitis**). The reasons for the different outcomes of an infection with the virus is yet to be resolved.

A number of other arenaviruses are also of clinical concern to humans. These viruses include the Lassa virus (the cause of Lassa fever), Junin virus (the cause of Argentine hemorrhagic fever), Machupo virus (the cause of Bolivian hemorrhagic fever), and Guanarito virus (the cause of Venezuelan hemorrhagic fever). **Hemorrhagic fevers** are characterized by copious bleeding, particularly of internal organs. The death rate in an outbreak of these hemorrhagic fevers can be extremely high.

An arenavirus is transmitted to a human via the urine or feces of the infected rodent. The urine or feces may contaminate food or water, may accidentally contact a cut on the skin, or the virus may be inhaled from dried feces. In addition, some arenaviruses can also be transmitted from one infected person to another person. Examples of such viruses are the Lassa virus and the Machupo virus. Person-to-person transmission can involve direct contact or contact of an

infected person with food implements or medical equipment, as examples.

As with other hemorrhagic fevers, treatment consists of stabilizing the patient. A **vaccine** for the Junin virus, which consists of living but weakened virus, has been developed and has been tested in a small cohort of volunteers. The results of these tests have been encouraging. Another vaccine, to the Lassa virus, consists of a protein component of the viral envelope. Tests of this vaccine in primates have also been encouraging to researchers.

Currently, the human illnesses caused by arenaviruses are best dealt with by the implementation of a rodent control program in those regions that are known to be sites of outbreaks of arenavirus illness. Because the elimination of rodents in the wild is virtually impossible, such a program is best directed at keeping the immediate vicinity of dwellings clean and rodent-free.

See also Hemorrhagic fevers and diseases; Virology, viral classification, types of viruses; Zoonoses

ARMILLARIA OSTOYAE

Armillaria ostoyae is a fungus, and is also known as the honey mushroom. The species is particularly noteworthy because of one fungus in the eastern woods of Oregon that is so far the biggest organism in the world.

Armillaria ostoyae grows from a spore by extending filaments called rhizomorphs into the surrounding soil. The rhizomorphs allow access to nutrients. The bulk of the fungus is comprised of these mycelial filaments. The filaments can also be called **hyphae**. The fungal hyphae can consist of cells each containing a **nucleus**, which are walled off from one another. Or, the cells may not be walled off, and a filament is essentially a long cell with multiple nuclei dispersed throughout its length.

For the giant fungus, using an average growth rate of the species as a gauge, scientists have estimated that the specimen in the Malheur National Forest in Oregon has been growing for some 2400 years. The growth now covers 2200 acres, an area equivalent to 1665 football fields.

Analysis of the genetic material obtained from different regions of the fungal growth has shown the **DNA** to be identical, demonstrating that the growth is indeed from the same fungus. The weight of the gigantic fungus has not been estimated.

As the giant fungus has grown the rhizomorph growth has penetrated into the interior of the tree. The fungus than draws off nutrients, suffocating the tree. As well, the mycelia can extend as deep as 10 feet into the soil, and can invade the roots of trees. When viewed from the air, the pattern of dead trees looks remarkably like a mushroom. The outline of the fungal boundary is 3.5 miles in diameter.

Scientists are studying the fungus because of the tree-killing ability it displays. Understanding more of the nature of this effect could lead to the use of the fungus to control tree growth.

The bulk of the gigantic fungus is some three feet underground. The only surface evidence of the fungus are periodic displays of golden mushrooms that are present in rainy times of the year.

Although not as well studied as the Oregon giant, another *Armillaria ostoyae* found in Washington state is even larger. Estimates put the area covered by the Washington state fungus at over 11000 acres.

See also Fungi

ASEXUAL GENERATION AND REPRODUCTION

Sexual reproduction involves the production of new cells by the fusion of sex cells (sperm and ova) to produce a genetically different cell. Asexual reproduction, on the other hand, is the production of new cells by simple division of the parent cell into two daughter cells (called binary fission). Because there is no fusion of two different cells, the daughter cells produced by asexual reproduction are genetically identical to the parent cell. The adaptive advantage of asexual reproduction is that organisms can reproduce rapidly, thus enabling the quick colonization of favorable environments.

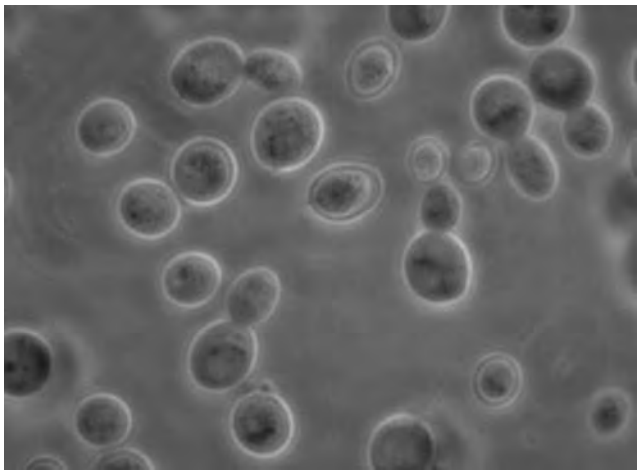
Duplication of organisms, whether sexually or asexually, involves the partitioning of the genetic material (**chromosomes**) in the cell **nucleus**. During asexual reproduction, the chromosomes divide by mitosis, which results in the exact duplication of the genetic material into the nuclei of the two daughter cells. Sexual reproduction involves the fusion of two gamete cells (the sperm and ova), each of which has half the normal number of chromosomes, a result of reduction division known as meiosis.

Bacteria, cyanobacteria, algae, **protozoa**, **yeast**, dandelions, and flatworms all reproduce asexually. When asexual reproduction occurs, the new individuals are called clones, because they are exact duplicates of their parent cells. Mosses reproduce by forming runners that grow horizontally, produce new stalks, then decompose, leaving a new plant that is a clone of the original.

Bacteria reproducing asexually double their numbers rapidly, approximately every 20 minutes. This reproduction rate is offset by a high death rate that may be the result of the accumulation of alcohol or acids that concentrate from the bacterial colonies.

Yeasts reproduce asexually by budding; they can also reproduce sexually. In the budding process a bulge forms on the outer edge of the yeast cell as nuclear division takes place. One of these nuclei moves into the bud, which eventually breaks off completely from the parent cell. Budding also occurs in flatworms, which divide into two and then regenerate to form two new flatworms.

Bees, ants, wasps, and other insects can reproduce sexually or asexually. In asexual reproduction, eggs develop without fertilization, a process called parthenogenesis. In some



Budding yeast cells undergoing asexual reproduction; yeast are single-celled fungi.

species the eggs may or may not be fertilized; fertilized eggs produce females, while unfertilized eggs produce males.

There are a number of crop plants that are propagated asexually. The advantage of asexual propagation to farmers is that the crops will be more uniform than those produced from seed. Some plants are difficult to cultivate from seed and asexual reproduction in these plants makes it possible to produce crops that would otherwise not be available for commercial marketing. The process of producing plants asexually is called vegetative propagation and is used for such crops as potatoes, bananas, raspberries, pineapples, and some flowering plants used as ornamentals. Farmers plant the so-called "eyes" of potatoes to produce duplicates of the parent. With banana plants, the suckers that grow from the root of the plant are separated and then planted as new ones. With raspberry bushes, branches are bent and covered with soil. They then grow into a separate plant with their own root system and can eventually be detached from the parent plant.

See also Cell cycle and cell division

ASILOMAR CONFERENCE

Soon after American microbiologist Hamilton Smith's 1970 discovery of the first restriction enzyme, it became possible to combine **DNA** from different sources into one molecule, producing recombinant DNA. Concern by scientists and lay people that some of this recombinant-technology DNA might be harmful to humans prompted the research to stop until scientists could evaluate its risks.

In February 1975 over 100 internationally respected molecular biologists met at the Asilomar conference center in California. There they decided upon a set of guidelines to be followed by all scientists doing recombinant DNA research. They considered every class of experiment and assigned each a level of risk: minimal, low, moderate, or high. Each level of risk required a corresponding set of containment procedures designed to minimize the chance of vectors (carriers) containing recombinant DNA molecules from escaping into the environment, where they could potentially harm humans or other parts of the ecosystem. Because these projected experiments had never been done, assignment to a risk category was, of course, somewhat speculative and subjective. Accordingly, the potential risks were arrived at by estimate.

At all risk levels, the guidelines called for the use of biological barriers. Bacterial host cells should be from strains unable to survive in natural environments (outside the test tube). Vectors carrying recombinant DNA, including **plasmids**, bacteriophages, and other **viruses**, were to be nontransmissible and unable to survive in natural environments.

For experiments having minimal risk, the guidelines recommended that scientists follow general microbiology safety procedures. These included not eating, drinking, or smoking in the lab; wearing laboratory coats in the work area; and promptly disinfecting contaminated materials.

Low-risk procedures required a bit more caution. For example, procedures producing aerosols, such as using a blender, were to be performed under an enclosed ventilation hood to eliminate the risk of the recombinant DNA being liberated into the air.

Moderate-risk experiments required the use of a laminar flow hood, the wearing of gloves, and the maintenance of negative air pressure in the laboratory. This would ensure that air currents did not carry recombinant DNA out of the laboratory.

Finally, in high-risk experiments, maximum precautions were specified. These included isolation of the laboratory from other areas by air locks, having researchers shower and change their clothing upon leaving the work area, and the incineration of exhaust air from the hoods.

Certain types of experiments were not to be done at all. These most potentially dangerous experiments included the **cloning** of recombinant DNA from highly pathogenic organisms or DNA containing toxin genes. Also forbidden were experiments involving the production of more than 10 liters of **culture** using recombinant DNA molecules that might render the products potentially harmful to humans, animals, or plants.

The scientists at the Asilomar conference also resolved to meet annually to re-evaluate the guidelines. As new procedures were developed and safer vectors and bacterial cells became available, it became possible to re-evaluate and relax some of the initially stringent and restrictive safety standards.

See also Recombination; Viral genetics; Viral vectors in gene therapy

ATOMIC FORCE MICROSCOPE

In 1985, the development of the atomic force **microscope** (AFM) allowed scientists to visualize the surface of cellular structures during some physiological processes. Along with the use of field ion microscopes and powerful scanning tunneling microscopes (STM), these advances in microscopy represent the most fundamental greatest advances since the development of the **electron microscope**.

Invented by Gerd Binnig and Christoph Gerber in Zurich, Switzerland, and Calvin Quate (1923–) in California, the AFM uses a tiny needle made of diamond, tungsten, or silicon, much like those used in the STM. While the STM relies upon a subject's ability to conduct electricity through its needle, the AFM scans its subjects by actually lightly touching them with the needle. Like that of a phonograph record, the AFM's needle reads the bumps on the subject's surface, rising as it hits the peaks and dipping as it traces the valleys. Of course, the topography read by the AFM varies by only a few molecules up or down, so a very sensitive device must be used to detect the needle's rising and falling. In the original model, Binnig and Gerber used an STM to sense these movements. Other AFM's later used a fine-tuned laser. The AFM has already been used to study the supermicroscopic structures of living cells and other objects that could not be viewed with the STM.

American physicist Paul Hansma (1946–) and his colleagues at the University of California, Santa Barbara, conduct various studies using AFM research. In 1989, this team succeeded in observing the blood-clotting process within blood cells. Hansma's team presented their findings in a 33–minute movie, assembled from AFM pictures taken every ten seconds. Other scientists are utilizing the AFM's ability to remove samples of cells without harming the cell structure. By adding a bit more force to the scanning needle, the AFM can scrape cells, making it the world's most delicate dissecting tool.

Scientists continue to apply this method to the study of living cells, particularly fragile structures on the cell surface, whose fragility makes them nearly impossible to view without distortion.

See also Bacterial membranes and cell wall; Bacterial surface layers; Bacterial ultrastructure; Microscope and microscopy

ATTENUATION • *see* VACCINE

ATTRACTANTS AND REPELLENTS

Attractants and repellents are compounds that stimulate the directed movement of **microorganisms**, in particular **bacteria**, towards or away from the compound. The directed movement in response to the presence of the attractant or repellent compound is a feature of a bacterial behavior known as chemotaxis.

Various compounds can act as attractants. Overwhelmingly, these are nutrients for the bacterium. Attractant compounds include sugars, such as maltose, ribose, galactose, and amino acids such as L-aspartate and L-serine.

Similarly, various compounds will cause a bacterium to move away. Examples of repellents include metals that are damaging or lethal to a bacterium (e.g., cobalt, nickel), membrane-disruptive compounds such as indole, and weak acids, which can damage the integrity of the cell wall.

The presence and influence of attractants and repellents on the movement of bacteria has been known for over a century. In the 1880s experiments demonstrated that bacteria would move into capillary tubes filled with meat extract and away from capillaries filled with acids.

Now, the molecular underpinning for this behavior is better understood. The chemotaxis process has been particularly well-studied in the related Gram-negative bacteria ***Escherichia coli*** and ***Salmonella*** *typhimurium*.

These bacteria are capable of self-propelled movement, by virtue of whip-like structures called flagella. Movement consists typically of a random tumbling interspersed with a brief spurt of directed movement. During the latter the bacterium senses the environment for the presence of attractants or repellents. If an attractant is sensed, the bacterium will respond by exhibiting more of the directed movement, and the movement will over time be in the direction of the attractant. If the bacterium senses a repellent, then the periods of directed movement will move the bacterium away from the compound. Both of these phenomena require mechanisms in the bacterium that can sense the presence of the compounds and can compare the concentrations of the compounds over time.

The detection of attractants and repellents is accomplished by proteins that are part of the cytoplasmic, or inner, membrane of bacteria such as *Escherichia coli* and *Salmonella typhymurium*. For example, there are four proteins that span the inner membrane, from the side that contacts the **cytoplasm** to the side that contacts the periplasmic space. These proteins are collectively called the methyl-accepting chemotaxis proteins (MCPs). The MCPs can bind different attractant and repellent compounds to different regions on their surface. For example, on of the MCPs can bind the attractants aspartate and maltose and the repellents cobalt and nickel.

The binding of an incoming attractant or repellent molecule to a MCP causes the addition or removal of a phosphate group to another molecule that is linked to the MCP on the cytoplasm side. Both events generate a signal that is transmitted to other bacterial mechanisms by what is known as a cascade. One of the results of the cascade is the control of the rotation of the flagella, so as to propel the bacterium forward or to generate the random tumbling motion.

The cascade process is exceedingly complex, with at least 50 proteins known to be involved. The proteins are also involved in other sensory events, such as to **pH**, temperature, and other environmental stresses.

The memory of a bacterium for the presence of an attractant or repellent is governed by the reversible nature of the binding of the compounds to the bacterial sensor proteins. The binding of an attractant or a repellent is only for a short time. If the particular compound is abundant in the environment, another molecule of the attractant or repellent will bind very soon after the detachment of the first attractant or repellent from the sensor. However, if the concentration of the attractant or repellent is decreasing, then the period between when the sensor-binding site becomes unoccupied until the binding of the next molecule will increase. Thus, the bacterium will have a gauge as to whether its movement is carrying the cell towards or away from the detected compound. Then, depending on whether the compound is desirable or not, corrections in the movement of the bacterium can be made.

See also Bacterial movement; Heat shock response

AUTOCLAVE • *see* STEAM PRESSURE STERILIZER

AUTOIMMUNITY AND AUTOIMMUNE DISORDERS

Autoimmune diseases are conditions in which a person's **immune system** attacks the body's own cells, causing tissue destruction. Autoimmune diseases are classified as either general, in which the autoimmune reaction takes place simultaneously in a number of tissues, or organ specific, in which the autoimmune reaction targets a single organ. Autoimmunity is accepted as the cause of a wide range of disorders, and is suspected to be responsible for many more. Among the most common diseases attributed to autoimmune disorders are rheumatoid arthritis, systemic lupus erythematosis (lupus), multiple sclerosis, myasthenia gravis, pernicious anemia, and scleroderma.

To further understand autoimmune disorders, it is helpful to understand the workings of the immune system. The purpose of the immune system is to defend the body against attack by infectious microbes (germs) and foreign objects. When the immune system attacks an invader, it is very specific—a particular immune system cell will only recognize and target one type of invader. To function properly, the immune system must not only develop this specialized knowledge of individual invaders, but it must also learn how to recognize and not destroy cells that belong to the body itself. Every cell carries protein markers on its surface that identifies it in one of two ways: what kind of cell it is (e.g., nerve cell, muscle cell, blood cell, etc.) and to whom that cell belongs. These markers are called **major histocompatibility complexes (MHCs)**. When functioning properly, cells of the immune system will not attack any other cell with markers identifying it as belonging to the body. Conversely, if the immune system cells do not recognize the cell as "self," they attach themselves to it and put out a signal that the body has been invaded, which in turn stimulates the production of substances such as antibodies that engulf and destroy the foreign particles. In case of autoimmune disorders, the immune system cannot distinguish between self cells and invader cells. As a result, the same destructive operation is carried out on the body's own cells that would normally be carried out on **bacteria**, **viruses**, and other such harmful entities.

The reason why the immune system become dysfunctional is not well understood. Most researchers agree that a combination of genetic, environmental, and hormonal factors play into autoimmunity. The fact that autoimmune diseases run in families suggests a genetic component. Recent studies have identified an antiphospholipid **antibody** (APL) that is believed to be a common thread among family members with autoimmune diseases. Among study participants, family members with elevated APL levels showed autoimmune disease, while those with other autoantibodies did not. Family members with elevated APL levels also manifested different forms of autoimmune disease, suggesting that APL may serve as a common trigger for different autoimmune diseases. Further study of the genetic patterns among unrelated family groups with APL suggests that a single genetic defect resulting in APL production may be responsible for several different autoimmune diseases. Current research focuses on finding an established APL inheritance pattern, as well as finding the autoimmune **gene** responsible for APL production.

A number of tests can help diagnose autoimmune diseases; however the principle tool used by physicians is antibody testing. Such tests involve measuring the level of antibodies found in the blood and determining if they react with specific antigens that would give rise to an autoimmune reaction. An elevated amount of antibodies indicates that a humoral immune reaction is occurring. Elevated antibody levels are also seen in common infections. These must be ruled out as the cause for the increased antibody levels. The antibodies can also be typed by class. There are five classes of antibodies and they can be separated in the laboratory. The class IgG is usually associated with autoimmune diseases. Unfortunately, IgG class antibodies are also the main class of antibody seen in normal immune responses. The most useful antibody tests involve introducing the patient's antibodies to samples of his or her own tissue—if antibodies bind to the tissue it is diagnostic for an autoimmune disorder. Antibodies from a person without an autoimmune disorder would not react to self tissue. The tissues used most frequently in this type of testing are thyroid, stomach, liver, and kidney.

Treatment of autoimmune diseases is specific to the disease, and usually concentrates on alleviating symptoms rather than correcting the underlying cause. For example, if a gland involved in an autoimmune reaction is not producing a hormone such as insulin, administration of that hormone is required. Administration of a hormone, however, will restore the function of the gland damaged by the autoimmune disease. The other aspect of treatment is controlling the inflammatory and proliferative nature of the immune response. This is generally accomplished with two types of drugs. Steroid compounds are used to control **inflammation**. There are many different steroids, each having side effects. The proliferative nature of the immune response is controlled with immunosuppressive drugs. These drugs work by inhibiting the replication of cells and, therefore, also suppress non-immune cells leading to side effects such as anemia. Prognosis depends upon the pathology of each autoimmune disease.

See also Antigens and antibodies; Antibody formation and kinetics; Antibody-antigen, biochemical and molecular reactions; Immune system; Immunity, cell mediated; Immunity, humoral regulation; Immunologic therapies; Immunosuppressant drugs; Major histocompatibility complex (MHC)

AUTOLOGOUS BANKING

Autologous banking is the recovery and storage of an individual's own blood. The blood can be from the circulating blood,

and is obtained in the same way that blood is obtained during blood donation procedures. As well, blood can be recovered from the umbilical cord following the birth of an infant. In both cases, the blood is stored for future use by the individual or for the extraction of a particular form of cell known as the stem cell.

Blood and blood products (e.g., plasma) can be stored in frozen form for extended periods of time without degrading. Thus, autologous banking represents a decision by an individual to maintain his/her blood in the event of a future mishap.

One motivation for autologous blood banking can be the increased assurance that the blood that will be used in subsequent operations or blood transfusions is free of contaminating **microorganisms** (e.g., **HIV**, **hepatitis**, etc.). Regardless, even with blood screening technologies there are still several hundred thousand transfusion-associated cases of hepatitis in the United States each year. From an immunological viewpoint, another reason for autologous banking is that autologous blood will be immunologically identical to the blood present at the time of return transfusion. This eliminates the possibility of an immune reaction to blood that is antigenically different from the individual's own blood.

The autologous blood collected from the umbilical cord is a source of stem cells. Stem cells are cells that have not yet undergone differentiation into the myriad of cell types that exist in the body (e.g., red blood cells, white blood cells, tissue cells), and so retain the ability to differentiate. Thus, under appropriate conditions, stem cells can be encouraged to differentiate into whatever target cell is desired. Although this reality has not yet been fully realized, the potential of stem cell technology as a therapy for various diseases has been demonstrated.

Umbilical cord blood cells also offer the advantage of being a closer match immunologically between individuals. The differences in blood cells between individuals due to the so-called major **histocompatibility** antigen is not as pronounced in cord blood cells. Thus, umbilical cord blood cells and tissue can be used for donation and transplantation. In addition, cord blood from closely related individuals can be pooled without inducing an immune response upon the use of the blood.

See also Antibody and antigen; Histocompatibility; Immunity, cell mediated

AUTOTROPHIC BACTERIA

An autotroph is an organism able to make its own food. Autotrophic organisms take inorganic substances into their bodies and transform them into organic nourishment. Autotrophs are essential to all life because they are the primary producers at the base of all food chains. There are two categories of autotrophs, distinguished by the energy each uses to synthesize food. Photoautotrophs use light energy; chemoautotrophs use chemical energy.

Photoautotrophic organisms (e.g., green plants) have the capacity to utilize solar radiation and obtain their energy directly from sunlight.

Until recently, scientists held there existed only a few kinds of **bacteria** that used chemical energy to create their own food. Some of these bacteria were found living near vents and active volcanoes on the lightless ocean floor. The bacteria create their food using inorganic sulfur compounds gushing out of the vents from the hot interior of the planet.

In 1993, scientists found many new species of **chemoautotrophic bacteria** living in fissured rock far below the ocean floor. These bacteria take in carbon dioxide and water and convert the chemical energy in sulfur compounds to run metabolic processes that create carbohydrates and sugars. A unique characteristic of these chemoautotrophic bacteria is that they thrive at temperatures high enough to kill other organisms. Some scientists assert that these unique bacteria should be classified in their own new taxonomic kingdom.

See also Bacterial kingdoms; Biogeochemical cycles; Extremophiles

AVERY, OSWALD THEODORE (1877-1955)

Canadian-born American immunologist

Oswald Avery was one of the founding fathers of **immunochemistry** (the study of the chemical aspects of **immunology**) and a major contributor to the scientific **evolution** of microbiology. His studies of the *Pneumococcus* virus (causing acute **pneumonia**) led to further classification of the virus into many distinct types and the eventual identification of the chemical differences among various pneumococci viral strains. His work on capsular polysaccharides and their role in determining immunological specificity and virulence in pneumococci led directly to the development of diagnostic tests to demonstrate circulating **antibody**. These studies also contributed to the development of therapeutic sera used to treat the pneumonia virus. Among his most original contributions to immunology was the identification of complex carbohydrates as playing an important role in many immunological processes. Avery's greatest impact on science, however, was his discovery that **deoxyribonucleic acid** (**DNA**) is the molecular basis for passing on genetic information in biological self-replication. This discovery forced geneticists of that time to reevaluate their emphasis on the protein as the major means of transmitting hereditary information. This new focus on DNA led to **James Watson** and **Francis Crick**'s model of DNA in 1952 and an eventual revolution in understanding the mechanisms of heredity at the molecular level.

Avery was born Halifax, Nova Scotia, to Joseph Francis and Elizabeth Crowdy Avery. His father was a native of England and a clergyman in the Baptist church, with which Avery was to maintain a lifelong affiliation. In 1887 the Avery family immigrated to the United States and settled in New York City, where Avery was to spend nearly sixty-one years of his life. A private man, he guarded his personal life, even from his colleagues, and seldom spoke of his past. He stressed that research should be the primary basis of evaluation for a scientific life, extending his disregard for personal matters to the point that he once refused to include details of a colleague's

personal life in an obituary. Avery's argument was that knowledge of matters outside of the laboratory have no bearing on the understanding of a scientist's accomplishments. As a result, Avery, who never married, managed to keep his own personal affairs out of the public eye.

Avery graduated with a B.A. degree from Colgate University in 1900, and he received his M.D. degree from Columbia University's College of Physicians and Surgeons in 1904. He then went into the clinical practice of general surgery for three years, soon turned to research, then became associate director of the bacteriology division at the Hoagland Laboratory in Brooklyn. Although his time at the laboratory enabled him to study species of **bacteria** and their relationship to infectious diseases, and was a precursor to his interest in immunology, much of his work was spent carrying out what he considered to be routine investigations. Eventually Rufus Cole, director of the Rockefeller Institute hospital, became acquainted with Avery's research, which included work of general bacteriological interest, such as determining the optimum and limiting hydrogen-ion concentration for *Pneumococcus* growth, developing a simple and rapid method for differentiating human and bovine *Streptococcus hemolyticus*, and studying bacterial nutrition. Impressed with Avery's analytical capabilities, Cole asked Avery to join the institute hospital in 1913, where Avery spent the remainder of his career.

At the institute Avery teamed up with A. Raymond Dochez in the study of the pneumococci (pneumonia) **viruses**, an area that was to take up a large part of his research efforts over the next several decades. Although Dochez eventually was to leave the institute, he and Avery maintained a lifelong scientific collaboration. During their early time together at the Rockefeller Institute, the two scientists further classified types of pneumococci found in patients and carriers, an effort that led to a better understanding of *Pneumococcus* lung infection and of the causes, incidence, and distribution of lobar pneumonia. During the course of these immunological classification studies, Avery and Dochez discovered specific soluble substances of *Pneumococcus* during growth in a cultured medium. Their subsequent identification of these substances in the blood and urine of lobar pneumonia patients showed that the substances were the result of a true metabolic process and not merely a result of disintegration during cell death.

Avery was convinced that the soluble specific substances present in pneumococci were somehow related to the immunological specificity of bacteria. In 1922, working with Michael Heidelberger and others at Rockefeller, Avery began to focus his studies on the chemical nature of these substances and eventually identified polysaccharides (complex carbohydrates) as the soluble specific substances of *Pneumococcus*. As a result, Avery and colleagues were the first to show that carbohydrates were involved in immune reactions. His laboratory at Rockefeller went on to demonstrate that these substances, which come from the cell wall (specifically the capsular envelopes of the bacteria), can be differentiated into several different serological types by virtue of the various chemical compositions depending on the type of *Pneumococcus*. For example, the polysaccharide in type 1 pneumococci contains nitrogen and is partly composed of galacturonic acid. Both types 2 and 3 pneumococci contain nitrogen-free carbohydrates as their soluble substances, but the carbohydrates in type 2 are made up mainly of glucose and those of type 3 are composed of aldobionic acid units. Avery and Heidelberger went on to show that these various chemical substances account for bacterial specificity. This work opened up a new era in biochemical research, particularly in establishing the immunologic identity of the cell.

In addition to clarifying and systemizing efforts in bacteriology and immunology, Avery's work laid the foundation for modern immunological investigations in the area of antigens (parts of proteins and carbohydrates) as essential molecular markers that stimulate and, in large part, determine the success of immunological responses. Avery and his colleagues had found that specific anti-infection antibodies worked by neutralizing the bacterial capsular polysaccharide's ability to interfere with **phagocytosis** (the production of immune cells that recognize and attack foreign material). Eventually Avery's discoveries led scientists to develop immunizations that worked by preventing an antigenic response from the capsular material. Avery also oversaw studies that showed similar immunological responses in *Klebsiella* pneumonia and *Hemophilus* **influenza**. These studies resulted in highly specific diagnostic tests and preparation of immunizing antigens and therapeutic sera. The culmination of Avery's work in this area was a paper he coauthored with **Colin Munro MacLeod** and **Maclyn McCarty** in 1944 entitled "Studies on the Chemical Nature of the Substance Inducing **Transformation** of Pneumococcal Types. Induction of Transformation by a Desoxyribonucleic Fraction Isolated from *Pneumococcus* Type III." In their article, which appeared in the *Journal of Experimental Medicine,* the scientists provided conclusive data that DNA is the molecular basis for transmitting genetic information in biological self-replication.

In 1931 Avery's focus turned to transformation in bacteria, building on the studies of microbiologist Frederick Griffith showing that viruses could transfer virulence. In 1928 Griffith first showed that heat-killed virulent pneumococci could make a nonvirulent strain become virulent (produce disease). In 1932 Griffith announced that he had manipulated immunological specificity in pneumococci. At that time Avery was on leave suffering from Grave's disease. He initially denounced Griffith's claim and cited inadequate experimental controls. But in 1931, after returning to work, Avery began to study transmissible hereditary changes in immunological specificity, which were confirmed by several scientists. His subsequent investigations produced one of the great milestones in biology.

In 1933 Avery's associate, James Alloway, had isolated a crude solution of the transforming agent. Immediately the laboratory's focus turned to purifying this material. Working with type-3 capsulated *Pneumococcus*, Avery eventually succeeded in isolating a highly purified solution of the transforming agent that could pass on the capsular polysaccharides's hereditary information to noncapsulated strains. As a result the noncapsulated strains could now produce capsular polysaccharides, a trait continued in following generations. The substance responsible for the transfer of genetic information was

DNA. These studies also were the first to alter hereditary material for treatment purposes.

Avery, however, remained cautious about the implications of the discovery, suspecting that yet another chemical component of DNA could be responsible for the phenomenon. But further work by McCarty and Moses Kunitz confirmed the findings. While some scientists, such as **Peter Brian Medawar**, hailed Avery's discovery as the first step out of the "dark ages" of genetics, others refused to give up the long-held notion that the protein was the basis of physical inheritance. The subsequent modeling of the DNA molecule by James Watson and Francis Crick led to an understanding of how DNA replicates, and demonstration of DNA's presence in all animals produced clear evidence of its essential role in heredity.

See also Antibody-antigen, biochemical and molecular reactions; Antibody and antigen; Antibody formation and kinetics; History of immunology; Immunogenetics; Immunologic therapies

AZOTOBACTER

The genus *Azotobacter* is comprised of **bacteria** that require the presence of oxygen to grow and reproduce, and which are inhabitants of the soil. There are six species of *Azotobacter*. The representative species is *Azotobacter vinelandii*.

The bacteria are rod-shaped and stain negative in the **Gram staining** procedure. Some species are capable of directed movement, by means of a flagellum positioned at one end of the bacterium. Furthermore, some species produce pigments, which lend a yellow-green, red-violet, or brownish-black hue to the soil where they are located.

Relative to other bacteria, *Azotobacter* is very large. A bacterium can be almost the same size as a **yeast** cell, which is a eucaryotic single-celled microorganism.

Azotobacter has several features that allow it to survive in the sometimes harsh environment of the soil. The bacteria can round up and thicken their cell walls, to produce what is termed a cyst. A cyst is not dormant, like a spore, but does allow the bacterium to withstand conditions that would otherwise be harmful to an actively growing vegetative cell. When in a cyst form, *Azotobacter* is not capable of nitrogen fixation. The second environmentally adaptive feature of the bacterium is the large amounts of slime material that can be secreted to surround each bacterium. Slime naturally retains water. Thus, the bacterium is able to sequester water in the immediate vicinity.

A noteworthy feature of *Azotobacter* is the ability of the bacteria to "fix" atmospheric nitrogen, by the conversion of this elemental form to ammonia. Plants are able to utilize the ammonia as a nutrient. Furthermore, like the bacteria *Klebsiella pneumoniae* and *Rhizobium leguminosarum*, *Azotobacter vinelandii* is able to accomplish this chemical conversion when the bacteria are living free in the soil. In contrast to *Rhizobium leguminosarum*, however, *Azotobacter vinelandii* cannot exist in an association with plants.

Azotobacter can accomplish nitrogen fixation by using three different **enzymes**, which are termed nitrogenases. The enzyme diversity, and an extremely rapid metabolic rate (the highest of any known living organism) allow the bacterium to fix nitrogen when oxygen is present. The other nitrogen-fixing bacteria possess only a single species of nitrogenase, which needs near oxygen-free conditions in order to function. The enhanced versatility of *Azotobacter* makes the microbe attractive for agricultural purposes.

See also Aerobes; Nitrogen cycle in microorganisms; Soil formation, involvement of microorganisms

B

B CELLS OR B LYMPHOCYTES

B lymphocytes, also known as B cells, are one of the five types of white blood cells, or leukocytes, that circulate throughout the blood. They and **T-lymphocytes** are the most abundant types of white blood cells. B lymphocytes are a vital part of the body's **immune system**. They function to specifically recognize a foreign protein, designated as an **antigen**, and to aid in destroying the invader.

B lymphocytes are produced and mature in the bone marrow. The mature form of the cell is extremely diverse, with a particular B cell being tailored to recognize just a single antigen. This recognition is via a molecule on the surface of the B cell, called a B cell receptor. There are thousands of copies of the identical receptor scattered over the entire surface of a B cell. Moreover, there are many thousands of B cells, each differing in the structure of this receptor. This diversity is possible because of rearrangement of genetic material to generate genes that encode the receptors. The myriad of receptors are generated even before the body has been exposed to the protein antigen that an individual receptor will recognize. B cells thus are one means by which our immune system has "primed" itself for a rapid response to invasion.

The surface receptor is the first step in a series of reactions in the body's response to a foreign antigen. The receptor provides a "lock and key" fit for the target antigen. The antigen is soluble; that is, floating free in the fluid around the B cell. A toxin that has been released from a bacterium is an example of a soluble antigen. The binding of the antigen to the B cell receptor triggers the intake of the bound antigen into the B cell, a process called receptor-mediated endocytosis. Inside the cell the antigen is broken up and the fragments are displayed one the surface of the B cell. These are in turn recognized by a receptor on the surface of a T lymphocyte, which binds to the particular antigen fragment. There follows a series of reactions that causes the B cell to differentiate into a plasma cell, which produces and secretes large amounts of an **antibody** to the original protein antigen.

Plasma cells live in the bone marrow. They have a limited lifetime of from two to twelve weeks. Thus, they are the immune system's way of directly addressing an antigen threat. When the threat is gone, the need for plasma cells is also gone. But, B lymphocytes remain, ready to differentiate into the antibody–producing plasma cells when required.

Within the past several years, research has indicated that the deliberate depletion of B cells might aid in thwarting the progression of autoimmune disease—where the body's immune system reacts against the body's own components—and so bring relief from, for example, rheumatoid arthritis. However, as yet the data is inconclusive, and so this promising therapy remains to be proven.

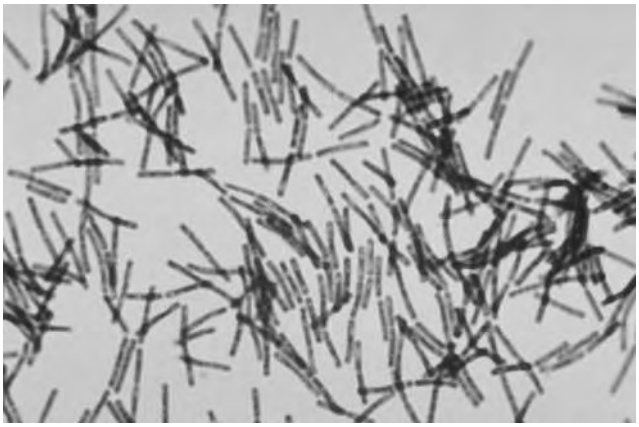
See also Antibody and antigen; Antibody formation and kinetics; Immunity, active, passive and delayed; Immunity, cell mediated; Immunization

BACILLUS THURINGIENSIS, INSECTICIDE

Bacillus thuringiensis is a Gram-positive rod-shaped bacterium. This bacterium is most noteworthy because of its use to kill butterfly and moth caterpillars (Lepidoptera), the larvae of mosquitoes, and some species of black fly, that are a damage to economically important plants or a health threat.

The basis of the bacterium's insecticidal power is a protein endotoxin (an endotoxin is a toxin that remains inside the bacterium). More correctly in terms of the lethal activity, the toxin is actually a so-called protoxin. That is, the molecule must be processed to some other form before the toxic activity is present.

Inside the bacterium the protoxin molecules collect together to form a crystal. These crystals are visible as two pyramids associated with each other when the **bacteria** are examined in light microscopy. Often the bacteria contain a bright spot under light microscopic illumination. This spot is an endospore (a spore that is contained within the bacterium).



Light microscope image of Bacillus bacteria.

The presence of an endospore is very useful. Like the spores of other bacterial species, the endospore of *Bacillus thuringiensis* allows the organism to survive inhospitable conditions in a dormant state. Endospores that contain the protoxin crystal can be applied to fields via crop-dusting aircraft.

The protoxin crystal is a hardy structure, and does not readily dissolve. However, in the gut of insects, where the **pH** is very basic, the protoxin can go into solution. When this happens an insect enzyme splits the molecule. One of the toxin fragments, the delta endotoxin, confers the lethal effect to the insect.

The delta endotoxin binds to the epithelial cells lining the gut wall of the insect. By creating holes in the cells, the toxin destroys the functioning of the gut, and causes massive cell death. The larva is unable to eat. Another consequence of the destruction is a modification of the pH to a more neutral level that is hospitable for the germination of the endospores. The resuscitation and growth of *Bacillus thuringiensis* within the insect gut kills the larva.

The use of *Bacillus thuringiensis* as an insecticide has been practiced since the 1930s. In the recent three or four decades, with the advent of techniques of molecular rearrangement, the specificity of the bacterium for target insect pests has been refined. These products now represent some one percent of the worldwide use of **fungicides**, herbicides and insecticides.

See also Bacteriocidal, bacteriostatic

BACTEREMIC

Bacteremic is a term that refers to the ability of a bacterium to multiply and cause an infection in the bloodstream. The invasion of the bloodstream by the particular type of **bacteria** is also referred to as bacteremia.

If the invading bacteria also release toxins into the bloodstream, the malady can also be called blood poisoning or septicemia. *Staphylococcus* and *Streptococcus* are typically associated with septicemia.

The bloodstream is susceptible to invasion by bacteria that gain entry via a wound or abrasion in the protective skin overlay of the body, or as a result of another infection elsewhere in the body, or following the introduction of bacteria during a surgical procedure or via a needle during injection of a drug.

Depending on the identity of the infecting bacterium and on the physical state of the human host (primarily with respect to the efficiency of the **immune system**), bacteremic infections may not produce any symptoms. However, some infections do produce symptoms, ranging from an elevated temperature, as the immune system copes with the infection, to a spread of the infection to the heart (endocarditis or pericarditis) or the covering of nerve cells (**meningitis**). In more rare instances, a bacteremic infection can produce a condition known as septic shock. The latter occurs when the infection overwhelms the ability of the body's defense mechanisms to cope. Septic shock can be lethal.

Septicemic infections usually result from the spread of an established infection. Bacteremic (and septicemic) infections often arise from bacteria that are normal resident on the surface of the skin or internal surfaces, such as the intestinal tract epithelial cells. In their normal environments the bacteria are harmless and even can be beneficial. However, if they gain entry to other parts of the body, these so-called commensal bacteria can pose a health threat. The entry of these commensal bacteria into the bloodstream is a normal occurrence for most people. In the majority of people, however, the immune system is more than able to deal with the invaders. If the immune system is not functioning efficiently then the invading bacteria may be able to multiply and establish an infection. Examples of conditions that compromise the immune system are another illness (such as acquired **immunodeficiency** syndrome and certain types of cancer), certain medical treatments such as irradiation, and the abuse of drugs or alcohol.

Examples of bacteria that are most commonly associated with bacteremic infections are *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Haemophilus*, and ***Escherichia coli***.

The generalized location of bacteremia produces generalized symptoms. These symptoms can include a fever, chills, pain in the abdomen, nausea with vomiting, and a general feeling of ill health. Not all these symptoms are present at the same time. The nonspecific nature of the symptoms may not prompt a physician to suspect bacteremia until the infection is more firmly established. Septic shock produces more drastic symptoms, including elevated rates of breathing and heartbeat, loss of consciousness and failure of organs throughout the body. The onset of septic shock can be rapid, so prompt medical attention is critical.

The discovery of bacteria in the blood should be taken as grounds to suspect bacteremia, because bacteria do not typically populate blood. Antibiotic therapy is usually initiated immediately, even if other options, such as the transient entry of bacteria from a cut, have actually occurred. In addition, antibiotic therapy is prudent because many bacteremic infections arise because of an ongoing infection elsewhere in the body. Along with the prompt start of treatment, the antibiotic used must be selected with care. Use of an ineffective antibi-

otic can provide the bacteria with enough time to undergo explosive increases in number, whereas the use of an antibiotic to which the bacteria are susceptible can quickly quell a brewing infection.

As with many other infections, bacteremic infections can be prevented by observance of proper hygienic procedures including hand washing, cleaning of wounds, and cleaning sites of injections to temporarily free the surface of living bacteria. The rate of bacteremic infections due to surgery is much less now than in the past, due to the advent of sterile surgical procedures, but is still a serious concern.

See also Bacteria and bacterial infection; Infection and resistance

BACTERIA AND BACTERIAL INFECTION

Infectious diseases depend on the interplay between the ability of pathogens to invade and/or proliferate in the body and the degree to which the body is able to resist. If the ability of a microorganism to invade, proliferate, and cause damage in the body exceeds the body's protective capacities, a disease state occurs. Infection refers to the growth of **microorganisms** in the body of a host. Infection is not synonymous with disease because infection does not always lead to injury, even if the pathogen is potentially virulent (able to cause disease). In a disease state, the host is harmed in some way, whereas infection refers to any situation in which a microorganism is established and growing in a host, whether or not the host is harmed.

The steps of pathogenesis, the progression of a disease state, include entry, colonization, and growth. Pathogens like bacteria use several strategies to establish virulence. The bacteria must usually gain access to host tissues and multiply before damage can be done. In most cases this requires the penetration of the skin, mucous membranes, or intestinal epithelium, surfaces that normally act as microbial barriers. Passage through the skin into subcutaneous layers almost always occurs through wounds and in rare instances pathogens penetrate through unbroken skin.

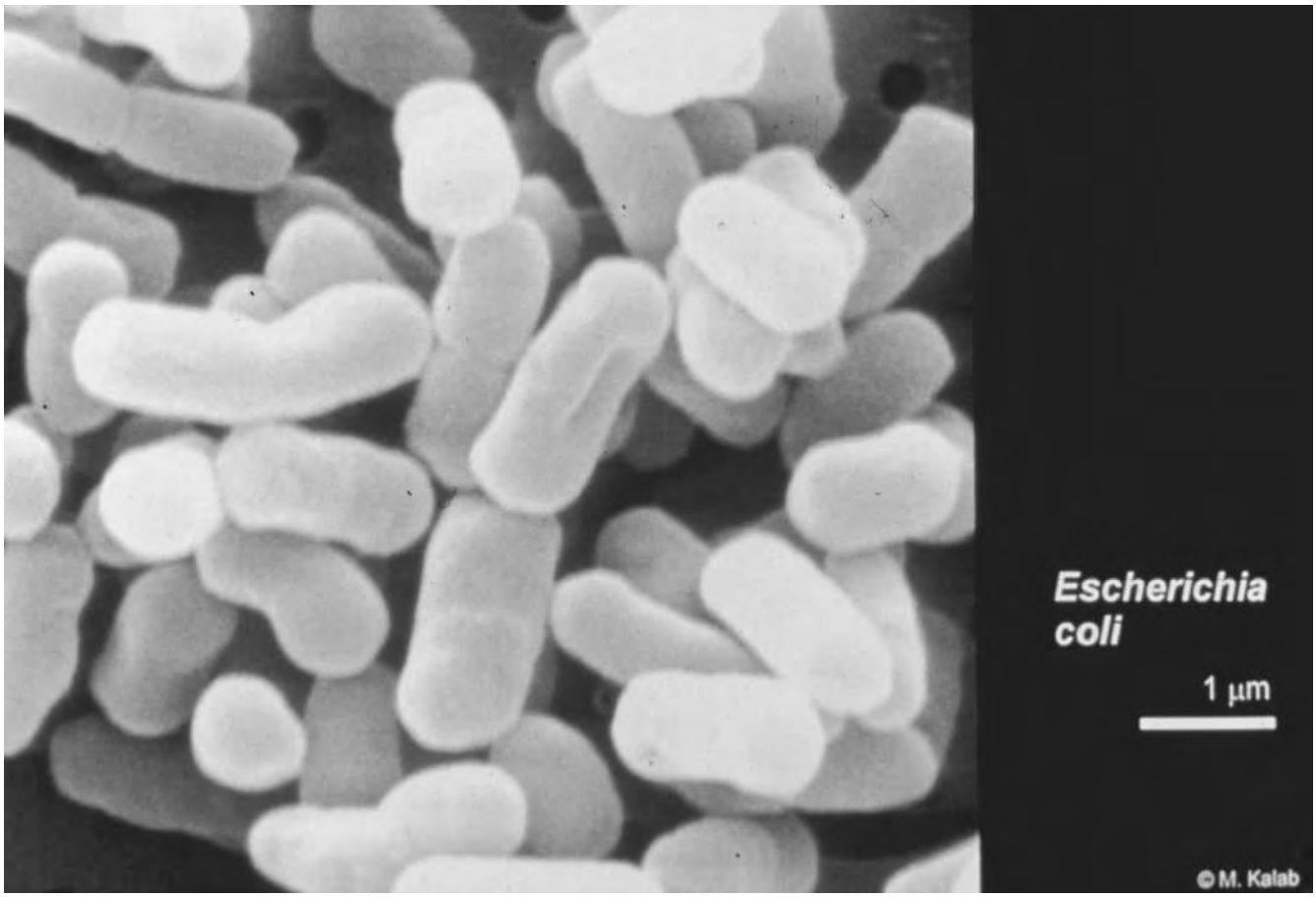
Most infections begin with the adherence of bacteria to specific cells on the mucous membranes of the respiratory, alimentary, or genitourinary tract. Many bacteria possess surface macromolecules that bind to complementary acceptor molecules on the surfaces of certain animal cells, thus promoting specific and firm adherence. Certain of these macromolecules are polysaccharides and form a meshwork of fibers called the **glycocalyx**. This can be important for fixing bacteria to host cells. Other proteins are specific, e.g., M-proteins on the surface of *Streptococcus pyogenes* which facilitate binding to the respiratory mucosal receptor. Also structures known as fimbrae may be important in the attachment process. For example, the fimbrae of *Neiseria gonorrhoeae* play a key role in the attachment of this organism to the urogenital epithelium where it causes a **sexually transmitted disease**. Also, it has been shown that fimbriated strains of ***Escherichia coli*** are much more frequent causes of urinary tract infections than strains lacking fimbrae, showing that these structures can indeed promote the capacity of bacteria to cause infection.

The next stage of infection is invasion that is the penetration of the epithelium to generate pathogenicity. At the point of entry, usually at small breaks or lesions in the skin or mucosal surfaces, growth is often established in the submucosa. Growth can also be established on intact mucosal surfaces, especially if the normal flora is altered or eliminated. Pathogen growth may also be established at sites distant from the original point of entry. Access to distant, usually interior, sites occurs through the blood or lymphatic system.

If a pathogen gains access to tissues by adhesion and invasion, it must then multiply, a process called colonization. Colonization requires that the pathogen bind to specific tissue surface receptors and overcome any non-specific or immune host defenses. The initial inoculum is rarely sufficient to cause damage. A pathogen must grow within host tissues in order to produce disease. If a pathogen is to grow, it must find appropriate nutrients and environmental conditions in the host. Temperature, **pH** and reduction potential are environmental factors that affect pathogen growth, but the availability of microbial nutrients in host tissues is most important. Not all nutrients may be plentiful in different regions. Soluble nutrients such as sugars, amino acids and organic acids may often be in short supply and organisms able to utilize complex nutrient sources such as glycogen may be favored. Trace elements may also be in short supply and can influence the establishment of a pathogen. For example, iron is thought to have a strong influence on microbial growth. Specific iron binding proteins called transferrin and lactoferrin exist in human cells and transfer iron through the body. Such is the affinity of these proteins for iron, that microbial iron deficiency may be common and administration of a soluble iron salt may greatly increase the virulence of some pathogens. Many bacteria produce iron-chelating compounds known as siderophores, which help them to obtain iron from the environment. Some iron chelators isolated from pathogenic bacteria are so efficient that they can actually remove iron from host iron binding proteins. For example, a siderophore called aerobactin, produced by certain strains of *E. coli* and encoded by the Col V plasmid, readily removes iron bound to transferring.

After initial entry, the organism often remains localized and multiplies, producing a small focus of infection such as a boil, carbuncle or pimple. For example, these commonly arise from Staphylococcus infections of the skin. Alternatively, the organism may pass through the lymphatic vessels and be deposited in lymph nodes. If an organism reaches the blood, it will be distributed to distal parts of the body, usually concentrating in the liver or spleen. Spread of the pathogen through the blood and lymph systems can result in generalized (systemic) infection of the body, with the organism growing in a variety of tissues. If extensive **bacterial growth** in tissues occurs, some of the organisms may be shed into the bloodstream, a condition known as bacteremia.

A number of bacteria produce extracellular proteins, which break down host tissues, encourage the spread of the organism and aid the establishment and maintenance of disease. These proteins, which are mostly **enzymes**, are called



Scanning electron micrograph of *Escherichia coli*, the cause of a gastrointestinal infection that can lead to severe complications.

virulence factors. For example, **streptococci**, **staphylococci** and pneumococci produce hyaluronidase, an enzyme that breaks down hyaluronic acid, a host tissue cement. They also produce proteases, nucleases and lipases that depolymerize host proteins, nucleic acids and fats. Clostridia that cause gas gangrene produce collagenase, and κ-toxin, which breaks down the collagen network supporting the tissues.

The ways in which pathogens bring about damage to the host are diverse. Only rarely are symptoms of a disease due simply to the presence of a large number of microorganisms, although a large mass of bacterial cells can block vessels or heart valves or clog the air passages of the lungs. In many cases, pathogenic bacteria produce toxins that are responsible for host damage. Toxins released extracellularly are called exotoxins, and these may travel from the focus of infection to distant parts of the body and cause damage in regions far removed from the site of microbial growth. The first example of an exotoxin to be discovered was the **diphtheria** toxin produced by *Corynebacterium diphtheriae*. Some Gram negative bacteria produce lipopolysaccharides as part of their cell walls, which under some conditions can be toxic. These are called endotoxins and have been studied primarily in the genera *Escherichia, Shigella,* and *Salmonella*.

See also Anti-adhesion methods; Antibiotic resistance, tests for; Immune system; Immunofluorescence; Immunology; Infection and resistance; Infection control

Bacteria, economic uses and benefits

• *see* Economic uses and benefits of microorganisms

Bacterial adaptation

Bacteria have been designed to be adaptable. Their surrounding layers and the genetic information for these and other structures associated with a bacterium are capable of alteration. Some alterations are reversible, disappearing when the particular pressure is lifted. Other alterations are maintained and can even be passed on to succeeding generations of bacteria.

The first antibiotic was discovered in 1929. Since then, a myriad of naturally occurring and chemically synthesized **antibiotics** have been used to control bacteria. Introduction of an antibiotic is frequently followed by the development of

resistance to the agent. Resistance is an example of the adaptation of the bacteria to the antibacterial agent.

Antibiotic resistance can develop swiftly. For example, resistance to **penicillin** (the first antibiotic discovered) was recognized almost immediately after introduction of the drug. As of the mid 1990s, almost 80% of all strains of *Staphylococcus aureus* were resistant to penicillin. Meanwhile, other bacteria remain susceptible to penicillin. An example is provided by Group A *Streptococcus pyogenes*, another Gram-positive bacteria.

The adaptation of bacteria to an antibacterial agent such as an antibiotic can occur in two ways. The first method is known as inherent (or natural) resistance. Gram-negative bacteria are often naturally resistant to penicillin, for example. This is because these bacteria have another outer membrane, which makes the penetration of penicillin to its target more difficult. Sometimes when bacteria acquire resistance to an antibacterial agent, the cause is a membrane alteration that has made the passage of the molecule into the cell more difficult. This is adaptation.

The second category of adaptive resistance is called acquired resistance. This resistance is almost always due to a change in the genetic make-up of the bacterial genome. Acquired resistance can occur because of mutation or as a response by the bacteria to the selective pressure imposed by the antibacterial agent. Once the genetic alteration that confers resistance is present, it can be passed on to subsequent generations. Acquired adaptation and resistance of bacteria to some clinically important antibiotics has become a great problem in the last decade of the twentieth century.

Bacteria adapt to other environmental conditions as well. These include adaptations to changes in temperature, **pH**, concentrations of ions such as sodium, and the nature of the surrounding support. An example of the latter is the response shown by *Vibrio parahaemolyticus* to growth in a watery environment versus a more viscous environment. In the more viscous setting, the bacteria adapt by forming what are called swarmer cells. These cells adopt a different means of movement, which is more efficient for moving over a more solid surface. This adaptation is under tight genetic control, involving the expression of multiple genes.

Bacteria react to a sudden change in their environment by expressing or repressing the expression of a whole lost of genes. This response changes the properties of both the interior of the organism and its surface chemistry. A well-known example of this adaptation is the so-called **heat shock response** of ***Escherichia coli***. The name derives from the fact that the response was first observed in bacteria suddenly shifted to a higher growth temperature.

One of the adaptations in the surface chemistry of Gram-negative bacteria is the alteration of a molecule called lipopolysaccharide. Depending on the growth conditions or whether the bacteria are growing on an artificial growth medium or inside a human, as examples, the lipopolysaccharide chemistry can become more or less water-repellent. These changes can profoundly affect the ability of antibacterial agents or immune components to kill the bacteria.

Another adaptation exhibited by *Vibrio parahaemolyticus*, and a great many other bacteria as well, is the formation of adherent populations on solid surfaces. This mode of growth is called a biofilm. Adoption of a biofilm mode of growth induces a myriad of changes, many involving the expression of previously unexpressed genes. As well de-activation of actively expressing genes can occur. Furthermore, the pattern of **gene** expression may not be uniform throughout the biofilm. Evidence from studies where the activity of living bacteria can be measured without disturbing the biofilm is consistent with a view that the bacteria closer to the top of the biofilm, and so closer to the outside environment, are very different than the bacteria lower down in the biofilm. A critical aspect of biofilms is the ability of the adherent bacteria to sense their environment and to convert this information into signals that trigger gene expression or inhibition.

Bacteria within a biofilm and bacteria found in other niches, such as in a wound where oxygen is limited, grow and divide at a far slower speed than the bacteria found in the test tube in the laboratory. Such bacteria are able to adapt to the slower growth rate, once again by changing their chemistry and gene expression pattern. When presented with more nutrients, the bacteria can often very quickly resume the rapid growth and division rate of their test tube counterparts. Thus, even though they have adapted to a slower growth rate, the bacteria remained "primed" for the rapid another adaptation to a faster growth rate.

A further example of adaptation is the phenomenon of chemotaxis, whereby a bacterium can sense the chemical composition of the environment and either moves toward an attractive compound, or shifts direction and moves away from a compound sensed as being detrimental. Chemotaxis is controlled by more than 40 genes that code for the production of components of the flagella that propels the bacterium along, for sensory receptor proteins in the membrane, and for components that are involved in signaling a bacterium to move toward or away from a compound. The adaptation involved in the chemotactic response must have a memory component, because the concentration of a compound at one moment in time must be compared to the concentration a few moments later.

See also Antiseptics; Biofilm formation and dynamic behavior; Evolution and evolutionary mechanisms; Mutations and mutagenesis

BACTERIAL APPENDAGES

A bacterial appendage protrudes outward from the surface of the microorganism. Some are highly anchored to the surface, whereas others, like the **glycocalyx**, are loosely associated with the surface.

The entire surface of a bacterium can be covered with glycocalyx (also known as the slime layer). The layer is made of chains of sugar. Protein can also be present. The exact chemical nature of a glycocalyx varies from one species of **bacteria** to another. A glycocalyx is easily identified in light microscopy by the application of India ink. The ink does not

penetrate the glycocalyx, which then appears as a halo around each bacteria.

A glycocalyx has a number of functions. It aids a bacterium in attaching to a surface. Surface contact triggers the production of a great deal of glycocalyx. The bacteria on the surface can become buried. This phenomenon has been well documented for *Pseudomonas aeruginosa*, which forms biofilms on surfaces in many environments, both within and outside of the body. The production of glycocalyx is a vital part of the biofilm formation.

By virtue of its chemical make-up, a glycocalyx will retain water near the bacteria, which protects the bacteria from drying out. Protection is also conferred against **viruses**, **antibiotics**, antibacterial agents such as detergents, and from the engulfing of the bacteria by immune macrophage cells (a process called **phagocytosis**). The mass of glycocalyx-enclosed bacteria becomes too large for a macrophage to engulf. For example, encapsulated strains of *Streptococcus pneumoniae* kill 90% of the animals it infects. Unencapsulated strains, however, are completely non-lethal. As another example of the protection conferred by the glycocalyx, *Pseudomonas aeruginosa* in an intact biofilm resist for hours concentrations of antibiotics up to one thousand times greater than those which kill within minutes their bacterial counterparts without glycocalyx and bacteria freed from the glycocalyx cocoon of the biofilm.

Glycocalyx material is easily removed from the bacterial surface. A glycocalyx that is more firmly anchored is known called as a capsule. Many disease causing bacteria tend to produce capsules when inside the human host, as a defense against phagocytosis.

Another type of bacterial appendage is the flagella (singular, flagellum). They appear as strings protruding outward from a bacterium. They are long, up to ten times the length of the bacterium. Each flagellum is composed of a spiral arrangement of a protein (flagellin). The flagella are closed off at the end removed from the cell. The end closest to the bacterial surface hooks into the membrane(s), where they are held by two structures termed basal bodies. The basal bodies act as bushings, allowing flagellar tube to turn clockwise and counterclockwise. By spinning around from this membrane anchor, flagella act as propellers to move a bacterium forward, or in a tumbling motion prior to a directed movement in the same or another forward path. These runs and tumbles enable a bacterium to move toward an attractant or away from a repellant. Generally termed taxis, these movements can be in response to nutrients (chemotaxis), oxygen (aerotaxis) or light (phototaxis). The tactic process is highly orchestrated, with sensory proteins detecting the signal molecule and conveying the signal into flagellar action.

Flagella are very powerful. They can propel bacteria at ten times their length per second. In contrast, an Olympic sprinter can propel himself at just over five body lengths per second. Depending upon the type of bacteria, flagella are characteristically arranged singly at only one end of the cell (monotrichous), singly at both ends of the cell (amphitrichous), in a tuft at one or a few sites (lophotrichous), or all over the bacterial surface (peritrichous).

The bacteria called **spirochetes** have a modified form of flagella, which is termed an endoflagella or an axial filament. In this case, the flagella is not an appendage, in that it is not external to the bacterium, but instead is found in the interior of the cell, running from one end of the cell to another. It is, however, similar in construction to flagella. Endoflagella attach to either end of a cell and provide the rigidity that aids a cell in turning like a corkscrew through its liquid environment.

Two other types of appendages are essentially tubes that stick out from the bacterial surface. The first of these is known as spinae (singular, spina). Spinae are cylinders that flare out at their base. They are a spiral arrangement of a single protein (spinin) that is attached only to the outer surface of the outer membrane. They have been detected in a marine pseudomonad and a freshwater bacterial species. Their formation is triggered by environmental change (**pH**, temperature, and sodium concentration). Once formed, spinae are extremely resilient, surviving treatment with harsh acids and bases. They are designed for longevity. Their function is unknown. Suggested functions include buoyancy, promoters of bacterial aggregation, and as a conduit of genetic exchange.

The appendages called pili are also tubes that protrude from the bacterial surface. They are smaller in diameter than spinae. Like spinae, pili are constructed of a protein (pilin). Unlike spinae, the functions of pili are well known. Relatively short pili are important in the recognition of receptors on the surface of a host cell and the subsequent attachment to the receptor. These are also known as fimbriae. There can be hundreds of fimbriae scattered all over the bacterial surface. Their attachment function makes fimbriae an important disease factor. An example is *Neiserria gonorrheae*, the agent of **gonorrhea**. Strains of the bacteria that produce fimbriae are more virulent than strains that do not manufacture the appendage. Not unexpectedly, such pili are a target of **vaccine** development. The second type of pili is called **conjugation** pili, sex pili, or F-pili. These are relatively long and only a few are present on a bacterium. They serve to attach bacteria together and serve as a portal for the movement of genetic material (specifically the circularly organized material called a plasmid) from one bacterium to the other. The genetic spread of **antibiotic resistance** occurs using pili.

See also Anti-adhesion methods; Bacteria and bacterial infection; Electron microscopic examination of microorganisms

BACTERIAL ARTIFICIAL CHROMOSOME (BAC)

Bacterial artificial **chromosomes** (BACs) involve a **cloning** system that is derived from a particular plasmid found in the bacterium ***Escherichia coli***. The use of the BAC allows large pieces of **deoxyribonucleic acid** (**DNA**) from bacterial or non-bacterial sources to be expressed in *Escherichia coli*. Repeated expression of the foreign DNA produces many copies in the bacterial cells, providing enough material for analysis of the

sequence of the DNA. BACs proved useful in the sequencing of the human genome.

The BAC is based on a plasmid in *Escherichia coli* that is termed the F (for fertility) plasmid. The F plasmid (or F factor) contains information that makes possible the process called **conjugation**. In conjugation, two *Escherichia coli* **bacteria** can physically connect and an exchange of DNA can occur.

A BAC contains the conjugation promoting genetic information as well as stretch of DNA that is destined for incorporation into the bacterium. The foreign DNA (e.g., portion of human genome) is flanked by sequences that mark the boundaries of the insert. The sequences are referred to as sequence tag connectors. When the BAC becomes incorporated into the genome of *Escherichia coli* the sequence tag connectors act as markers to identify the inserted foreign DNA.

Using a BAC, large stretches of DNA can be incorporated into the bacterial genome and subsequently replicated along with the bacterial DNA. In **molecular biology** terminology, pieces of DNA that contain hundreds of thousands of nucleotides (the building blocks of DNA) can be inserted into a bacterium at one time. As the process is done using different sections of the foreign DNA, the amount of DNA that can be analyzed can be very large.

BACs were developed in 1992. Since then, their usefulness has grown immensely. The primary reason for this popularity is the stability of the inserted DNA in the bacterial genome. Because the inserted DNA remains in the bacterial genome during repeated cycles of replication, the information is not lost. As well, the BAC can be sequenced using the normal tools of molecular biology.

The most dramatic recent example of the power of BACs is their use by **The Institute for Genomic Research** (**TIGR**) in the technique of **shotgun cloning** that was employed in the sequence determination of the human genome. Many fragments of the human genome could be incorporated into BACs. The resulting "library" could be expressed in *Escherichia coli* and the sequences determined. Subsequently, these sequences could be reconstructed to produce the orderly sequence of the actual genome. This approach proved to be less expensive and quicker than the method known as directed sequencing, where a genome was sequenced in a linear fashion starting at one end of the genome.

The total number of fragments of the DNA from the human genome that have been expressed in *Escherichia coli* by the use of BACs is now close to one million. In addition to the human genome, BACs have also been used to sequence the genome of agriculturally important plants such as corn and rice, and of animals such as the mouse.

With the realization of the sequence of the human genome, the use of BACs is becoming important in the screening of the genome for genetic abnormalities. Indeed, BAC cloning kits are now available commercially for what is termed genomic profiling.

See also Biotechnology; Plasmid and plastid

BACTERIAL EPIDEMICS • *see* EPIDEMICS, BACTERIAL

BACTERIAL FOSSILIZATION • *see* FOSSILIZATION OF BACTERIA

BACTERIAL GENETICS • *see* MICROBIAL GENETICS

BACTERIAL GROWTH AND DIVISION

The growth and division of **bacteria** is the basis of the increase of bacterial colonies in the laboratory, such as colony formation on **agar** in a liquid growth medium, in natural settings, and in infections.

A population of bacteria in a liquid medium is referred to as a **culture**. In the laboratory, where growth conditions of temperature, light intensity, and nutrients can be made ideal for the bacteria, measurements of the number of living bacteria typically reveals four stages, or phases, of growth, with respect to time. Initially, the number of bacteria in the population is low. Often the bacteria are also adapting to the environment. This represents the lag phase of growth. Depending on the health of the bacteria, the lag phase may be short or long. The latter occurs if the bacteria are damaged or have just been recovered from deep-freeze storage.

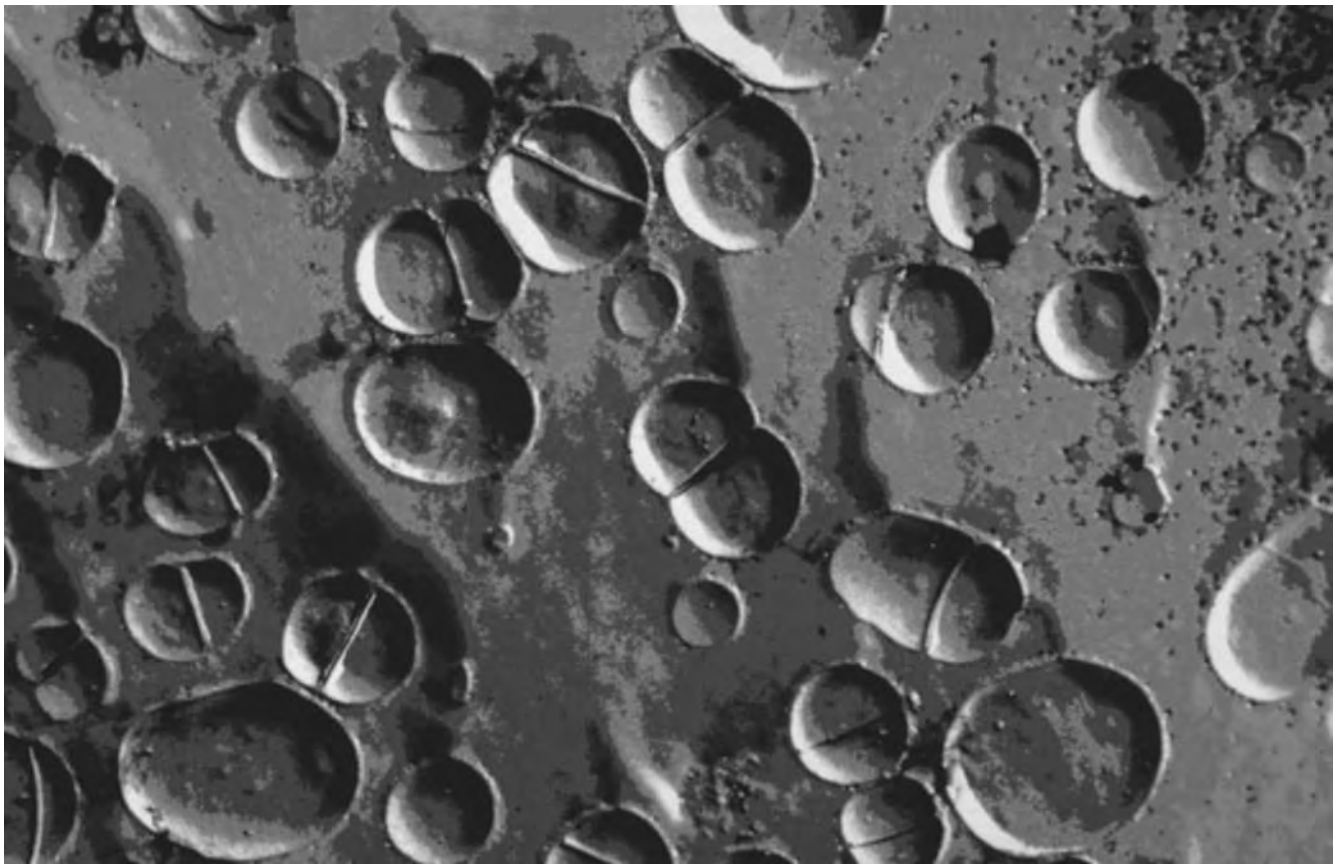
After the lag phase, the numbers of living bacteria rapidly increases. Typically, the increase is exponential. That is, the population keeps doubling in number at the same rate. This is called the log or logarithmic phase of culture growth, and is the time when the bacteria are growing and dividing at their maximum speed. For ***Escherichia coli***, for example, the rate of growth and division of a single bacterium (also called the generation time) during the log phase is 15 to 20 minutes. In the log phase, most of the bacteria in a population are growing and dividing.

The explosive growth of bacteria cannot continue forever in the closed conditions of a flask of growth medium. Nutrients begin to become depleted, the amount of oxygen becomes reduced, and the **pH** changes, and toxic waste products of metabolic activity begin to accumulate. The bacteria respond to these changes in a variety of ways to do with their structure and activity of genes. With respect to bacteria numbers, the increase in the population stops and the number of living bacteria plateaus. This plateau period is called the stationary phase. Here, the number of bacteria growing and dividing is equaled by the number of bacteria that are dying.

Finally, as conditions in the culture continue to deteriorate, the proportion of the population that is dying becomes dominant. The number of living bacteria declines sharply over time in what is called the death or decline phase.

Bacteria growing as colonies on a solid growth medium also exhibit these growth phases in different regions of a **colony**. For example, the bacteria buried in the oldest part of the colony are often in the stationary or death phase, while the bacteria at the periphery of the colony are in the actively dividing log phase of growth.

Culturing of bacteria is possible such that fresh growth medium can be added at rate equal to the rate at which culture is removed. The rate at which the bacteria grow is



Freeze fracture electron micrograph showing dividing Streptococcus bacteria. The division plane between the daughter cells is evident in some bacteria.

dependent on the rate of addition of the fresh medium. Bacteria can be tailored to grow relatively slow or fast and, if the set-up is carefully maintained, can be maintained for a long time.

Bacterial growth requires the presence of environmental factors. For example, if a bacterium uses organic carbon for energy and structure (chemoheterotrophic bacteria) then sources of carbon are needed. Such sources include simple sugars (glucose and fructose are two examples). Nitrogen is needed to make amino acids, proteins, lipids and other components. Sulphur and phosphorus are also needed for the manufacture of bacterial components. Other elements, such as potassium, calcium, magnesium, iron, manganese, cobalt and zinc are necessary for the functioning of **enzymes** and other processes.

Growth is also often sensitive to temperature. Depending on the species, bacteria exhibit a usually limited range in temperatures in which they can growth and reproduce. For example, bacteria known as mesophiles prefer temperatures from 20°–50° C (68°–122° F). Outside this range growth and even survival is limited.

Other factors, which vary depending on species, required for growth include oxygen level, pH, osmotic pressure, light and moisture.

The obvious events of growth and division that are apparent from measurement of the numbers of living bacteria are the manifestation of a number of molecular events. At the level of the individual bacteria, the process of growth and replication is known as binary division. Binary division occurs in stages. First, the parent bacterium grows and becomes larger. Next, the genetic material inside the bacterium uncoils from the normal helical configuration and replicates. The two copies of the genetic material migrate to either end of the bacterium. Then a cross-wall known as a septum is initiated almost precisely at the middle of the bacterium. The septum grows inward as a ring from the inner surface of the membrane. When the septum is complete, an inner wall has been formed, which divides the parent bacterium into two so-called daughter bacteria. This whole process represents the generation time.

Bacterial division is initiated by as-yet unidentified sensors of either the volume or the length of the bacterium. The sensors trigger a series of events, including the formation of the septum. In septum formation are number of proteins are recruited to the site where septal formation will begin. They may be guided to the site by the concentration of a trio of proteins that either inhibit or promote the formation of a so-called Z-ring. The Z-ring is analogous to a drawstring, and is likely an integral part of the inwardly growing septum wall.

Septum formation must be coordinated with other cellular events, such as genetic replication. As well, the growth of the cell wall is a coordinated process. The **peptidoglycan** is the stress-bearing structure of a bacterium. Therefore, the insertion of new material into the existing peptidoglycan must be done in such a way that the strength of the peptidoglycan network is maintained. Otherwise, the bacterium bursts.

While proteins important in bacterial growth and division have been identified, such as the Min series of proteins active in septum formation, the nature of their actions still remains unresolved.

See also Bacterial membranes and cell wall; Colony and colony formation

BACTERIAL GROWTH CURVE • *see* BACTERIAL GROWTH AND DIVISION

BACTERIAL KINGDOMS

Bacterial kingdoms are part of the classification scheme that fits **bacteria** into appropriate groupings based on certain criteria. The kingdom is the broadest classification category.

There are two kingdoms of prokaryotes. These are the bacteria (or **eubacteria**) and the archaebacteria (or the **Archaea**). The members of these two kingdoms appear similar in shape and appearance, even under the extreme magnification of the **electron microscope**. However, they are very different from each other in a number of molecular and biochemical aspects. It is these differences that have resulted in the **microorganisms** being grouped into separate kingdoms.

For example, eubacteria contain the rigid, stress-bearing network known as the **peptidoglycan**. The only exceptions are the bacteria from the genera *Mycoplasma* and *Chlamydia*. Archaebacteria do not contain peptidoglycan. Instead, they contain a different structure that is called pseudomurein.

Another major difference in the prokaryotic kingdoms is in the sequence of a species of **ribonucleic acid** (**RNA**) known as 16S ribosomal (r) RNA. The 16 S rRNA is found in many prokaryotic and eukaryotic cells. The function it performs is vital to the life of the cell. Hence, the RNA species has not been altered very much over evolutionary time. The 16s rRNA species of eubacteria and Archaebacteria are very different. Thus, these microorganisms must have taken different evolutionary paths long ago.

Within the eubacterial kingdom are other divisions also known as kingdoms. These divisions are again determined based on the differences in the sequences of the 16S rRNA of the various bacteria. These sequence differences within the eubacterial kingdom are, however, not as pronounced as the sequences differences between the eubacteria and Archaebacteria kingdoms.

The first eubacterial kingdom is referred to as protobacteria. This designation encompasses most of the bacteria that are Gram-negative. Because a great many bacteria are Gram-negative, the protobacterial kingdom is extremely diverse in the shape and the biochemical characteristics of the bacteria. Examples of protobacteria include the photosynthetic purple bacteria, *Pseudomonas*, and bacteria that dwell in the intestinal tract of warm-blooded animals (e.g., ***Escherichia coli***, *Salmonella*, and *Shigella*.

The second eubacterial kingdom is comprised of the Gram-positive bacteria. This group is also diverse in shape and chemical character. The kingdom is further split into two major groups, based on the proportion of the nucleic acid that is composed of two particular building blocks (guanosine and cytosine). One group contains those bacteria whose **DNA** is relatively low in G and C (e.g., *Clostridium*, *Staphylococcus*, *Bacillus*, **lactic acid bacteria**, Mycoplasma). The other group is made up of bacteria whose DNA is relatively enriched in G and C (e.g., *Actinomyces*, *Streptomyces*, *Bifidobacterium*. The latter group contains most of the antibiotic-producing bacteria that are known.

The various eubacterial kingdoms, and the Archaebacterial kingdom, are markedly different in 16S rRNA sequence from the eukaryotic kingdoms (plants, **fungi**, animals). Thus, following the establishment of these life forms, the **eukaryotes** began to diverge from the evolutionary paths followed by the eubacteria and Archaebacteria.

See also Life, origin of; Microbial taxonomy

BACTERIAL MEMBRANE TRANSPORT • *see* PROKARYOTIC MEMBRANE TRANSPORT

BACTERIAL MEMBRANES AND CELL WALL

Bacteria are bounded by a cell wall. The cell wall defines the shape of the microorganism, exerts some control as to what enters and exits the bacterium, and, in the case of infectious **microorganisms**, can participate in the disease process.

Many bacteria can be classified as either Gram-positive or Gram-negative. The Gram stain is a method that differentiates bacteria based on the structure of their cell wall. Gram-positive bacteria retain the crystal violet stain that is applied to the bacteria, and appear purple. In contrast, gram-negative bacteria do not retain this stain, but are "counterstained" red by the safranin stain that is applied later. The basis of these different staining behaviors lies in the composition of the cell walls of each Gram type.

Gram-positive bacteria have a cell wall that consists of a single membrane and a thick layer of **peptidoglycan**. Gram-negative bacteria have a cell wall that is made up of two membranes that sandwich a region known as the periplasmic space or **periplasm**. The outermost membrane is designated the outer membrane and the innermost one is known as the inner membrane. In the periplasm lies a thin peptidoglycan layer, which is linked with the overlaying outer membrane.

The cell wall of Gram-positive bacteria tends to be 2 to 8 times as thick as the Gram-negative wall.

When thin sections of bacteria are viewed in the transmission **electron microscope**, the membranes appear visually similar to a railroad track. There are two parallel thickly stained lines separated by an almost transparent region. The dark regions are the charged head groups of molecules called **phospholipids**. Bacterial phospholipids consist of the charged, hydrophilic ("water-loving") head region and an uncharged, **hydrophobic** ("water-hating") tail. The tail is buried within the membrane and forms most of the electron-transparent region evident in the electron microscope.

Phospholipids make up the bulk of bacterial membranes. In Gram-positive bacteria and in the inner membrane of Gram-negative bacteria the phospholipids are arranged fairly evenly on either "leaflet" of the membrane. In contrast, the outer membrane of Gram-negative bacteria is asymmetric with respect to the arrangement of phospholipids. The majority of the phospholipids are located at the inner leaflet of the membrane. The outer leaflet contains some phospholipid, and also proteins and a lipid molecule termed lipopolysaccharide.

The asymmetrical arrangement of the Gram-negative outer membrane confers various functions to the bacterium. Proteins allow the diffusion of compounds across the outer membrane, as long as they can fit into the pore that runs through the center of the protein. In addition, other proteins function to specifically transport compounds to the inside of the bacterium in an energy-dependent manner. The lipopolysaccharide component of the outer membrane is capable of various chemical arrangements that can influence the bacterium's ability to elude host immune defenses. For example, when free of the bacterium, lipopolysaccharide is referred to as endotoxin, and can be toxic to mammals, including humans.

The presence of the outer membrane makes the existence of the periplasm possible. The periplasm was once thought to be just functionless empty space. Now, however, the periplasm is now known to have very important functions in the survival and operation of the bacterium. The region acts as a **buffer** between the very different chemistries of the external environment and the interior of the bacterium. As well, specialized transport **proteins and enzymes** are located exclusively in this region. For example, the periplasm contains proteins that function to sense the environment and help determine the response of a bacterium to environmental cues, such as occurs in the directed movement known as chemotaxis.

Not all bacteria have such a cell wall structure. For example the bacteria known as mycobacteria lack a peptidoglycan and have different components in the cell membrane. Specifically, a compound called mycolic acid is present. Other bacteria called *Mycoplasma* lack a cell wall. They need to exist inside a host cell in order to survive.

The synthesis of the cell wall and the insertion of new cell wall material into the pre-existing wall is a highly coordinated process. Incorporation of the new material must be done so as not to weaken the existing wall. Otherwise, the bacterium would lose the structural support necessary for shape and survival against the osmotic pressure difference between the interior and exterior of the bacterium. Wall synthesis and insertion involves a variety of **enzymes** that function in both the mechanics of the process and as sensors. The latter stimulate production of the cell wall as a bacterium readies for division into two daughter cells.

See also Bacterial ultrastructure; Bacterial surface layers

BACTERIAL MOVEMENT

Bacterial movement refers to the self-propelled movement of **bacteria**. This movement is also referred to motility. The jiggling movement seen in some nonmotile bacteria that are incapable of self-propelled movement is due to the bombardment of the bacteria by water molecules. This so-called Brownian motion is not considered to represent bacterial movement.

There are several types of bacteria movement. The most common occurs by the use of appendages called flagella. A bacterium can contain a single flagellum, several flagella located at one or both poles of the cell, or many flagella dispersed all over the bacterial surface. Flagella can rotate in a clockwise or counterclockwise direction. When the motion is counterclockwise, even multiple flagella can unite into a flagellar bundle that functions as a propeller. This occurs when the bacterium is moving towards a chemical attractant or away from a repellent in the behavior known as chemotaxis. If the flagella turn in the opposite direction, the coordinated motion of the flagella stops, and a bacterium will "tumble," or move in an undirected and random way.

Spirochaete bacteria have flagella that are internal. These so-called axial filaments provide the rigidity that enables the spiral bacterium to twist around the axis of the filament. As a result, the bacterium literally screws itself through the fluid. Reversal of the twist will send the bacterium in a reverse direction. Examples of bacteria that move in this manner include *Treponema pallidum* and *Rhodospirillum rubrum*.

The bacteria that are known as **gliding bacteria** exhibit another type of bacterial movement. One example of a gliding bacterium is the cyanobacterium *Oscillatoria*. Gliding movement is exactly that; a constant gliding of a bacterium over a surface. The basis of this movement is still not clear, although it is known to involve a complex of proteins.

In a human host, disease causing bacteria such as *Salmonella typhymurium* can move along the surface of the host cells. This movement is due to another bacterial appendage called a pilus. A bacterium can have numerous pili on its surface. These hair-like appendages act to bind to surface receptors and, when retracted, pull the bacteria along the surface. Movement stops when a suitable area of the host cell surface is reached.

See also Bacterial appendages

BACTERIAL SHAPES • *see* BACTERIAL ULTRASTRUCTURE

BACTERIAL SMEARS • *see* MICROSCOPE AND MICROSCOPY

Bacterial surface layers

Bacterial surface layers are regularly arranged arrays, often comprised of the same component molecule, which are located on the surface of **bacteria**. The prototype surface layer is referred to as a S layer.

S layers are found on many bacteria that are recovered their natural environment, as well as on most of the known archaebacteria. Examples of bacteria that possess S layers include *Aeromonas salmonicida*, *Caulobacter crescentus*, *Deinococcus radiodurans*, *Halobacterium volcanii*, and *Sulfolobus acidocaldarius*. In many bacteria, the production of the surface layer proteins and assembly of the surface array ceases once the bacteria are cultured in the artificial and nutrient-rich conditions of most laboratory media.

The S layer of a particular bacterium is composed entirely of one type of protein, which self-assembles into the two-dimensional array following the extrusion of the proteins to the surface of the bacterium. The array visually resembles the strings of a tennis racket, except that the spaces between adjacent proteins are very small. In some Gram-positive bacteria the surface layer proteins are also associated with the rigid **peptidoglycan** layer than lies just underneath. The combination of the two layers confers a great deal of strength and support to the bacterium.

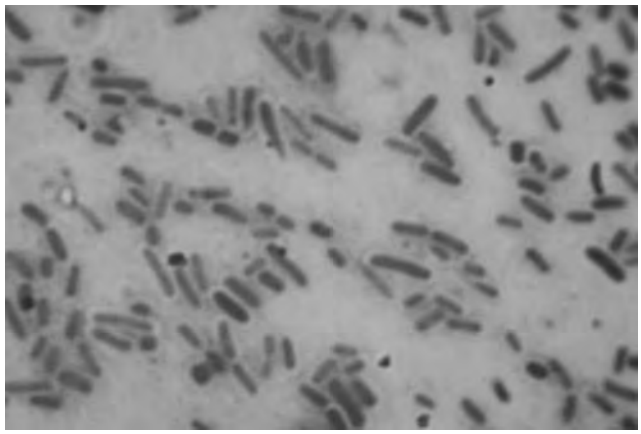
Bacterial surface layers are the outermost surface component of bacteria. As such, they modulate the interaction of the bacterium with its external environment, and are the first line of defense against antibacterial compounds. S layers, for example, act as sieves, by virtue of the size of the holes in between adjacent protein molecules. The layer can physically restrict the passage of molecules, such as destructive **enzymes**, that are larger than the pores. The S layer around the bacterium *Bdellovibrio bacteriovorans* even precludes attack from predators of the bacterium.

Some disease-causing bacteria possess S layers. These bacteria include *Corynebacterium diphtheriae* and *Bacillus anthracis*. Microscopic examination of bacteria found in the mouth has also revealed S layers. Possession of surface layers by these bacteria aids the bacteria in avoiding the process of **phagocytosis**. This is thought to be because the protein surface layer makes the bacteria more **hydrophobic** ("water hating") than bacteria of the same species that does not have the surface layer. The increasingly hydrophobic cells are not readily phagocytosed.

Bacterial ultrastructure

Bacterial ultrastructure is concerned with the cellular and molecular construction of **bacteria**. The bulk of research in bacterial ultrastructure investigates the ultrastructure of the cell wall that surrounds bacteria.

The study of bacterial ultrastructure began with the development of the staining regimen by Danish pathologist **Christian Gram** (1853–1938) that classifies the majority of bacteria as either Gram-negative or Gram-positive. The latter



Light micrograph of *Klebsiella* bacteria showing "halo" created by the capsule.

bacteria retain the crystal violet stain, while Gram-negative bacteria do not retain this stain and are stained by the second stain that is applied, safranin. While the basis for this difference was not known at first, scientists suspected that the structure of the wall surrounding the contents of the bacteria might be involved.

Subsequent to the time of Gram, scientists have discovered that the cell wall plays only a secondary role in the Gram stain reactions. However, the cell wall of Gram-positive bacteria is indeed much different than that of Gram-negative bacteria. The study of bacterial ultrastructure relates these constituent differences to the intact cell wall. In other words, ultrastructure explores the structure of each constituent and the chemical and other associations that exist between these constituents.

The exploration of bacterial ultrastructure requires samples that are as undisturbed as possible from their natural, or so-called native, state. This has been challenging, since much of the information that has been obtained has come from the use of electron microscopy. The techniques of conventional transmission electron microscopy and scanning electron microscopy require the removal of water from the sample. Because the bulk of living things, including bacteria, are comprised of water, the removal of this fluid can have drastic consequences on the structure of the bacteria. Much effort has gone into the development of regimens that chemically "fix" bacteria, so that structure is maintained following the subsequent removal of water.

Techniques have also been developed that prepare bacteria for transmission electron microscopy without the necessity of removing water from the specimen. One technique uses an embedding resin (a substance in which the bacteria are immersed and, when the resin is hardened, allows thin slices of the bacteria to be cut) that mixes with water. This resin is harder to work with than the conventional resins that are not water-soluble. Thus, while valuable information can be obtained using water-soluble resins, a great deal of experience is necessary to produce high quality results.

A second technique of sample preparation relies on the instantaneous freezing of the bacteria. Freezing is so fast that the interior water does not extensively crystallize (which would be extremely damaging to structure). Again, an experienced analyst can produce samples that information concerning the native ultrastructure of the bacteria.

In the past several decades, other tools are increasing the ultrastructure information that can be obtained. For example, the technique of atomic force microscopy can produce information on the atomic associations between adjacent molecules on the surface of bacteria. Atomic force microscopy has been very useful in ultrastructure studies of the regularly structured surface layers on bacteria.

Modern techniques of **molecular genetics** can also yield ultrastructure information. **Mutants** can be selected or designed in which a particular **gene** or genes has been rendered incapable of producing a protein product. If the gene is involved with cell wall constituents, the analysis of the wall can reveal the alterations that have occurred in the absence of the gene product. An example are the many mutants that are defective in the construction or assembly of lipopolysaccharide, a carbohydrate and lipid constituent of the outer membrane of Gram-negative bacteria. The loss of the carbohydrate portion of lipopolysaccharide makes the outer membrane more **hydrophobic**.

One approach that has been known for decades still yields useful information concerning bacterial ultrastructure. This is the substitution of the metals present in the cell wall with other metals. Metals act like glue to hold various wall components in association with one another. Examples of such metallic species include calcium and magnesium. Out-competing these species by supplying large concentrations of another metal, the influence of the normal metallic species can be assessed. For example, replacement of metals in the Gram-negative outer membrane can cause the release of lipopolysaccharide and the formation of bubbles along the surface of the membrane, where the underlying attachment to the rigid **peptidoglycan** layer is disrupted.

The use of specific antibodies to determine the molecular arrangement of ultrastructural constituent targets greatly enhances the effectiveness of agents to be used in drug therapy.

See also Atomic force microscope; Bacterial appendages; Bacterial surface layers; Caulobacter; Electron microscope, transmission and scanning; Electron microscopic examination of microorganisms; Sheathed bacteria

BACTERIOCHLOROPHYLL • *see* PHOTOSYNTHESIS

BACTERIOCIDAL, BACTERIOSTATIC

Bacteriocidal is a term that refers to the treatment of a bacterium such that the organism is killed. Bacteriostatic refers to a treatment that restricts the ability of the bacterium to grow. A bacteriocidal treatment is always lethal and is also referred to as **sterilization**. In contrast, a bacteriocidal treatment is necessarily lethal.

Bacteriocidal methods include heat, filtration, radiation, and the exposure to chemicals. The use of heat is a very popular method of sterilization in a microbiology laboratory. The dry heat of an open flame incinerates **microorganisms** like **bacteria**, **fungi** and **yeast**. The moist heat of a device like an autoclave can cause deformation of the protein constituents of the microbe, as well as causing the microbial membranes to liquefy. The effect of heat depends on the time of exposure in addition to form of heat that is supplied. For example, in an autoclave that supplies a temperature of 121° F (49.4° C), an exposure time of 15 minutes is sufficient to kill the so-called vegetative form of bacteria. However, a bacterial spores can survive this heat treatment. More prolonged exposure to the heat is necessary to ensure that the spore will not germinate into a living bacteria after autoclaving. The relationship between the temperature and the time of exposure can be computed mathematically.

A specialized form of bacteriocidal heat treatment is called **pasteurization** after **Louis Pasteur**, the inventor of the process. Pasteurization achieves total killing of the bacterial population in fluids such as milk and fruit juices without changing the taste or visual appearance of the product.

Another bacteriocidal process, albeit an indirect one, is filtration. Filtration is the physical removal of bacteria from a fluid by the passage of the fluid through the filter. The filter contains holes of a certain diameter. If the diameter is less than the smallest dimension of a bacterium, the bacterium will be retained on the surface of the filter it contacts. The filtered fluid is sterile with respect to bacteria. Filtration is indirectly bactericidal since the bacteria that are retained on the filter will, for a time, be alive. However, because they are also removed from their source of nutrients, the bacteria will eventually die.

Exposure to electromagnetic radiation such as ultraviolet radiation is a direct means of killing bacteria. The energy of the radiation severs the strands of **deoxyribonucleic acid** in many locations throughout the bacterial genome. With only one exception, the damage is so severe that repair is impossible. The exception is the radiation resistant bacterial genus called *Deinococcus*. This genus has the ability to piece together the fragments of **DNA** in their original order and enzymatic stitch the pieces into a functional whole.

Exposure to chemicals can be bacteriocidal. For example, the gas ethylene oxide can sterilize objects. Solutions containing alcohol can also kill bacteria by dissolving the membrane(s) that surround the contents of the cell. Laboratory benches are routinely "swabbed" with an ethanol solution to kill bacteria that might be adhering to the bench top. Care must be taken to ensure that the alcohol is left in contact with the bacteria for a suitable time (e.g., minutes). Otherwise, bacteria might survive and can even develop resistance to the bactericidal agent. Other chemical means of achieving bacterial death involve the alteration of the **pH**, salt or sugar concentrations, and oxygen level.

Antibiotics are designed to be bacteriocidal. **Penicillin** and its derivatives are bactericidal because they act on the **pep-**

tidoglycan layer of Gram-positive and Gram-negative bacteria. By preventing the assembly of the peptidoglycan, penicillin antibiotics destroy the ability of the peptidoglycan to bear the stress of osmotic pressure that acts on a bacterium. The bacterium ultimately explodes. Other antibiotics are lethal because they prevent the manufacture of DNA or protein. Unlike bacteriocidal methods such as the use of heat, bacteria are able to acquire resistance to antibiotics. Indeed, such resistance by clinically important bacteria is a major problem in hospitals.

Bacteriostatic agents prevent the growth of bacteria. Refrigeration can be bacteriostatic for those bacteria that cannot reproduce at such low temperatures. Sometimes a bacteriostatic state is advantageous as it allows for the long-term storage of bacteria. Ultra-low temperature freezing and lyophilization (the controlled removal of water from a sample) are means of preserving bacteria. Another bacteriocidal technique is the storage of bacteria in a solution that lacks nutrients, but which can keep the bacteria alive. Various buffers kept at refrigeration temperatures can keep bacteria alive for weeks.

See also Bacterial growth and division; Disinfection and disinfectants; Laboratory techniques in microbiology

BACTERIOLOGY • *see* BACTERIA AND BACTERIAL INFECTION

BACTERIOPHAGE AND BACTERIOPHAGE TYPING

A bacteriophage, or phage, is a virus that infects a bacterial cell, taking over the host cell's genetic material, reproducing itself, and eventually destroying the bacterium. The word phage comes from the Greek word *phagein*, meaning "to eat." Bacteriophages have two main components, protein coat and a nucleic acid core of **DNA** or **RNA**. Most DNA phages have double-stranded DNA, whereas phage RNA may be double or single-stranded. The **electron microscope** shows that phages vary in size and shape. Filamentous or threadlike phages, discovered in 1963, are among the smallest **viruses** known. Scientists have extensively studied the phages that infect ***Escherichia coli*** *(E.coli)*, **bacteria** that are abundant in the human intestine. Some of these phages, such as the T4 phage, consist of a capsid or head, often polyhedral in shape, that contains DNA, and an elongated tail consisting of a hollow core, a sheath around it, and six distal fibers attached to a base plate. When T4 attacks a bacterial cell, proteins at the end of the tail fibers and base plate attach to proteins located on the bacterial wall. Once the phage grabs hold, its DNA enters the bacterium while its protein coat is left outside.

Double stranded DNA phages reproduce in their host cells in two different ways: the lytic cycle and the lysogenic cycle. The lytic cycle kills the host bacterial cell. During the lytic cycle in *E.coli*, for example, the phage infects the bacterial cell, and the host cell commences to transcribe and translate the viral genes. One of the first genes that it translates encodes an enzyme that chops up the *E.coli* DNA. The host now follows instructions solely from phage DNA which commands the host to synthesize phages. At the end of the lytic cycle, the phage directs the host cell to produce the enzyme, lysozyme, that digests the bacterial cell wall. As a result, water enters the cell by osmosis and the cell swells and bursts. The destroyed or lysed cell releases up to 200 phage particles ready to infect nearby cells. On the other hand, the lysogenic cycle does not kill the bacterial host cell. Instead, the phage DNA is incorporated into the host cell's chromosome where it is then called a prophage. Every time the host cell divides, it replicates the prophage DNA along with its own. As a result, the two daughter cells each contain a copy of the prophage, and the virus has reproduced without harming the host cell. Under certain conditions, however, the prophage can give rise to active phages that bring about the lytic cycle.

In 1915, the English bacteriologist Frederick Twort (1877–1950) first discovered bacteriophages. While attempting to grow *Staphylococcus aureus*, the bacteria that most often cause boils in humans, he observed that some bacteria in his laboratory plates became transparent and died. Twort isolated the substance that was killing the bacteria and hypothesized that the agent was a virus. In 1917, the French-Canadian scientist **Felix H. d'Hérelle** independently discovered bacteriophages as well. The significance of this discovery was not appreciated, however, until about thirty years later when scientists conducted further bacteriophage research. One prominent scientist in the field was Salvador E. Luria (1912–1991), an Italian-American biologist especially interested in how x rays cause **mutations** in bacteriophages. Luria was also the first scientist to obtain clear images of a bacteriophage using an electron microscope. Salvador Luria emigrated to the United States from Italy and soon met Max Delbruck (1906–1981), a German-American molecular biologist. In the 1940s, Delbruck worked out the lytic mechanism by which some bacteriophages replicate. Together, Luria, Delbruck and the group of researchers that joined them studied the genetic changes that occur when viruses infect bacteria. Until 1952, scientists did not know which part of the virus, the protein or the DNA, carried the information regarding viral replication. It was then that scientists performed a series of experiments using bacteriophages. These experiments proved DNA to be the molecule that transmits the genetic information. (In 1953, the **Watson** and **Crick** model of DNA explained how DNA encodes information and replicates). For their discoveries concerning the structure and replication of viruses, Luria, Delbruck, and **Hershey** shared the Nobel Prize for physiology or medicine in 1969. In 1952, two American biologists, Norton Zinder and Joshua Lederberg at the University of Wisconsin, discovered that a phage can incorporate its genes into the bacterial chromosome. The phage genes are then transmitted from one generation to the next when the bacterium reproduces. In 1980, the English biochemist, Frederick Sanger, was awarded a Nobel Prize for determining the nucleotide sequence in DNA using bacteriophages.

In the last several decades, scientists have used phages for research. One use of bacteriophages is in genetic engineering, manipulating genetic molecules for practical uses. During genetic engineering, scientists combine genes from different sources and transfer the recombinant DNA into cells where it is expressed and replicated. Researchers often use *E. coli* as a host because they can grow it easily and the bacteria is well studied. One way to transfer the recombinant DNA to cells utilizes phages. Employing **restriction enzymes** to break into the phage's DNA, scientists splice foreign DNA into the viral DNA. The recombinant phage then infects the bacterial host. Scientists use this technique to create new medical products such as vaccines. In addition, bacteriophages provide information about genetic defects, human development, and disease. One geneticist has developed a technique using bacteriophages to manipulate genes in mice, while others are using phages to infect and kill disease-causing bacteria in mice. In addition, microbiologists found a filamentous bacteriophage that transmits the **gene** that encodes the toxin for cholera, a severe intestinal disease that kills tens of thousands worldwide each year.

See also Bacteria and bacterial infection; Biotechnology; Cell cycle (prokaryotic), genetic regulation of; Chromosomes, prokaryotic; Genetic regulation of prokaryotic cells; Laboratory techniques in microbiology; Phage genetics; Phage therapy; Viral genetics; Viral vectors in gene therapy; Virus replication; Viruses and responses to viral infection

BALTIMORE, DAVID (1938-)

American microbiologist

At the age of 37, David Baltimore was awarded the 1975 Nobel Prize in physiology or medicine for his groundbreaking work on retrovirus replication. Baltimore pioneered work on the **molecular biology** of animal **viruses**, especially poliovirus, and his investigations of how viruses interact with cells led, in 1970, to the discovery of a novel enzyme, reverse transcriptase. This enzyme transcribes **RNA** to **DNA** and permits a unique family of viruses, the **retroviruses**, to code for viral proteins. Baltimore shared the Nobel Prize with virologist Renato Dulbecco and oncologist Howard Temin, who independently discovered the same enzyme. Baltimore's achievement had profound implications for the scientific community because it challenged the central dogma of molecular biology, which stated that the flow of genetic information was unidirectional, running from DNA to RNA to proteins. His work also contributed to the understanding of certain diseases such as **AIDS**, now known to be caused by the retrovirus **HIV**.

David Baltimore was born in New York City to Richard Baltimore and Gertrude Lipschitz. Baltimore was a gifted science student while still in high school; he attended a prestigious summer program at the Jackson Laboratory in Bar Harbor, Maine, in which he studied mammalian genetics. It was during this program that he met his future colleague, Howard Temin, and decided to pursue a career in scientific research. As an undergraduate Baltimore attended Swarthmore College in Pennsylvania and graduated in 1960 with high honors in chemistry. He started graduate work at the Massachusetts Institute of Technology (MIT), but he transferred after one year to the Rockefeller Institute, now the Rockefeller University, in New York. There he studied with Richard M. Franklin, a molecular biophysicist specializing in RNA viruses. Baltimore earned his Ph.D. in 1964 and then completed three years postdoctoral research at the Salk Institute in La Jolla, California. There he met Renato Dulbecco, who developed innovative techniques for examining animal viruses, and **Alice Shih Huang**, who later became his wife. Huang was Baltimore's postdoctoral student at Salk, collaborated in some of his viral research, and later became a full professor at the Harvard Medical School. In 1968 Baltimore joined the MIT faculty, became full professor in 1972, and in 1973 was awarded a lifetime research professorship by the American Cancer Society. After winning the Nobel Prize in 1975 Baltimore continued to be honored for his work. He was elected to the National Academy of Sciences and the American Academy of Arts and Sciences in 1974.

In the mid-1970s Baltimore turned to research in molecular **immunology**, establishing a major presence in that rapidly developing field. As a prominent figure in the scientific community, Baltimore became outspoken about the potential risks of genetic engineering. He was concerned that the rapidly developing techniques of molecular biology might be misused. In 1975 Baltimore initiated a conference in which scientists attempted to design a self-regulatory system regarding experiments with recombinant DNA. In the following year the National Institutes of Health established a committee to oversee federally funded experiments in the field of genetic engineering. Baltimore became a key link between basic molecular biology and the burgeoning **biotechnology** industry. In 1984 he was appointed founding director of the new Whitehead Institute for Biomedical Research, which is affiliated with MIT; he remained at this post until 1990. In that position Baltimore made significant advances in the field of immunology and synthetic **vaccine** research. He earned wide admiration for forging dynamically amicable relations between the two institutions, developing a high-powered young faculty and molding the Whitehead into one of the world's leading institutions of its kind. Baltimore was a major influence in shaping the Human Genome Project and is an outspoken advocate of greater national investment in AIDS research.

In July 1990 Baltimore became president of Rockefeller University, launching an energetic program of fiscal and structural reform to bring the university's finances under control and to provide greater encouragement for junior faculty members. He resigned from the presidency at the end of 1991. At the time he was caught up in a controversy that stemmed from his support of a collaborator who had been charged with scientific misconduct, but whose scientific honesty he had resolutely defended. Several years later the collaborator was found to be innocent of all the charges raised against her. Baltimore remained on the faculty of Rockefeller University until 1994, when he returned to MIT as the Ivan R. Cottrell Professor of Molecular Biology and Immunology, and then Institute Professor.

During his career, David Baltimore has served on numerous governmental advisory committees. Apart from being a member of the National Academy of Sciences, he is also affiliated with the Pontifical Academy of Sciences, the American Academy of Arts and Sciences, and the Royal Society of London. At the end of 1996 he was appointed head of the newly created AIDS Vaccine Research Committee of the National Institutes of Health, a group that supports all efforts to accelerate the discovery of a vaccine against AIDS.

See also AIDS, recent advances in research and treatment; Immunogenetics; Viral genetics; Viral vectors in gene therapy; Viruses and responses to viral infection

BASIDOMYCETES

Basidomycetes are a fungal group belonging to the Eukarya domain, which includes all life forms composed by nucleated cells. Basidomycetes are classified under the **Fungi** kingdom as belonging to the phylum –mycota (i.e., Basidomycota or Basidiomycota), class –mycetes (i.e., Basidomycetes). Fungi are frequently **parasites** that decompose organic material from their hosts, such as those growing on rotten wood, although some may cause serious plant diseases such as smuts (Ustomycetes) and rusts (Teliomycetes). Some live in a symbiotic relationship with plant roots (Mycorrhizae). A cell type termed basidium is responsible for sexual spore formation in Basidomycetes, through nuclear fusion followed by meiosis, thus forming haploid basidiospores. Fungi pertaining to the Basidomycota phylum may present dikaryotic **hyphae**, i.e., walled filamentous cylindrical structures resembling branches that are formed when the two nuclei in the apical cell of a hypha divide simultaneously. One divides in the hyphal main axis and the other into the clamp, thus giving origin to a temporary monokaryotic clamp cell that is then fused to the sub apical cell, restoring the dikaryotic status. Spores are lined next to one another on the several neighboring basidia that form the Hymenium on the mushroom gill. Each spore usually bears the haploid product of meiosis. In adverse conditions, the spores may remain dormant for long periods, from months to years. When conditions are favorable, the spores germinate into uninucleated hyphae, forming monokaryotic mycelia. A dikaryotic **mycelium** is formed as the result of the fusion of two monokaryotic mycelia. Basidomycetes' sexual spores are more often than not disseminated through the wind, either by passive or forced spore discharge.

Basidomycetes comprises over 15,000 species, belonging to 15 different orders, most of them wood-rotting species. Some examples of Basidomycetes are as follows: Coral Fungus or Ramaria, pertaining to the Hymeniales order; Stinkhorn or Phallus, from the Phallales order; Corn smut or Ustilago, from the Ustilaginales order; Puffball or Lycoperdon, from the Lycoperdales order; White Button Pizza or *Agaricus bisporus,* from the Agaricales order.

The cell walls of fungi contain distinct layers, mainly constituted by **chitin** and not by cellulose. Multicellular fungi such as mushrooms have their vegetative bodies constituted mainly by filamentous hyphae. As parasites, Basidomycota and other fungi phyla (i.e., Chytridiomycota, Zygomycota, Ascomycota), do not itlize **photosynthesis**, and therefore, lack clorophyll. They produce instead several different exoenzymes, which are released directly on their hosts through invading filaments that can reach the target substance to be enzymatically decomposed. The exoenzymes are utilized in the digestion of the available organic substance from which they absorb micronutrients to synthesize and store great amounts of glycogen, whereas plants store energy under the form of starch. They also contain in their cell membranes ergosterol, a sterol found exclusively in fungi.

See also Chitin; Eukaryotes; Fungal genetics; Mycology

BATCH AND CONTINUOUS CULTURE • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

BAYER, MANFRED E. (1928-)

German physician

While educated as a physician, Manfred Bayer is best known for the series of fundamental contributions he has made to the study of bacterial and viral ultrastructure. He was the first person to visualize the **yellow fever** virus in cultured cells, and to obtain ultrathin sections of the changes caused to the cell wall of ***Escherichia coli*** by the antibiotic **penicillin**. The latter achievement helped guide the development of future **antibiotics** active against the bacterial wall. In the 1960s, he identified zones of adhesion between the inner and outer membranes of *Escherichia coli*. Bayer's rigorous experiments established that these adhesion zones that were apparent in thin sections of cells examined by the technique of transmission electron microscopy had biochemical significance e.g., routing of bacterial components to the surface of the cell, route for passage of **viruses** into the bacterium, specific site of certain enzyme activity). In recognition of his efforts, the adhesion sites were dubbed "Bayer's adhesion zones."

Bayer was born in Görlitz, Prussia (now Poland). Following his high school education he enrolled in the biology program at the University of Kiel in Germany. He obtained his degree in 1949. Following this, he was accepted for medical studies at the University of Hamburg, Germany. He completed his preclinical training in 1953 and clinical training in 1955. From 1957 to 1959 he studied physics at the same university. During this same period he earned his accreditation as a physician, and undertook research studies in pathology. This research led to a Research Associate position at the University of Hamburg from 1957 to 1961. Also during this period Bayer undertook diploma studies at the university's Institute of Tropical Medicine and Parasitology. He received his diploma in 1961.

From 1960 to 1962, Bayer was an Assistant Member of the Institute of Tropical Diseases and Parasitology. Then, he immigrated to the United States to take up the position of

Research Associate with The Institute of Cancer Research in Philadelphia. He has remained at the institute ever since, as an Assistant Member (1964–1967), Associate Member (1967–1978), Member (1978–1986), Senior Member (1986 to 1997), and Senior Member Emeritus (1997 to present). As well, he was an Adjunct Professor for Microbiology at the University of Pennsylvania Medical School (1971–2000) and a Honorary Visiting Professor at Dalhousie University, Halifax (1981–present).

Another contribution that Bayer has made to the field of **bacterial ultrastructure** is in the use of water-soluble embedding resins. The resins are used to solidify samples so that thin sections can be cut for **electron microscopic examination**. Some of the early refinements to the quality of the resins and the embedding techniques were pioneered by Bayer and his colleagues.

In 1968, Bayer and his colleagues deduced the structure of the structural units that form the **hepatitis** virus. Their discovery led to the formulation of a **vaccine**.

In addition to his research activities, Bayer has been a teacher and mentor to hundreds of students over four decades.

Bayer's research and teaching accomplishments have garnered him numerous honors and awards, including the Japanese Society for the Promotion of Science (1977), fellowship in the American Academy of Microbiology, and over 15 years as an editorial member of the *Journal of Bacteriology.*

See also Bacterial ultrastructure; Electron microscopic examination of microorganisms

BEAVER FEVER • *see* GIARDIA AND GIARDIASIS

BECKWITH, JONATHAN ROGER (1935-)

American microbiologist

Jonathan Roger Beckwith is the American Cancer Society Research Professor of Microbiology and **Molecular Genetics** at Harvard Medical School in Boston, Massachusetts. He is world renowned for his studies of bacterial **gene** expression, protein secretion, the structure and function of membrane proteins, and bacterial division. He has authored over 230 scientific publications. As well, Beckwith is a commentator of the societal aspects of science, with over 70 publications to date.

Beckwith was born and educated in the Boston area. He graduated from Newton High School in 1953 and went onto Harvard College, where he graduated in 1957 with an A.B. in Chemistry. From there, he attended Harvard University, graduating with a Ph.D. in Biochemical Sciences in 1961. From 1961 until 1965, he was a National Institutes of Health postdoctoral fellow in the laboratories of Arthur Pardee (Berkeley and Princeton), William Hayes (London), Sidney Brenner (Cambridge), and Francois Jacob (Paris). In 1965, he returned to Harvard as an Associate in the Department of Bacteriology and **Immunology**, the faculty he has remained with to this day (the name of the department was changed to Microbiology and Molecular Genetics in 1969). He became an Assistant Professor in 1966, an Associate Professor in 1968, and a Professor in 1969.

Beckwith's studies of protein expression, secretion, membrane dynamics and division in the bacterium ***Escherichia coli*** have been of fundamental importance in both basic bacteriology and in the development of clinical strategies to deal with *Escherichia coli* infections. As part of these studies, in 1969 Beckwith was the first person to isolate a gene.

In addition to his fundamental scientific research, Beckwith has also been an active commentator on the social impact of genetics, the need to present scientific issues and topics in language that is accessible to all, and on the political influences on scientific research.

The scope and importance of Beckwith's achievements in fundamental bacterial genetics and societal aspects of genetics have been recognized by his receipt of many awards and honors. These include a Merit Award from the National Institutes of Health (1986), the Eli Lilly Award for outstanding achievement in microbiology (1970), and the Genetics Society of America Medal (1993).

Beckwith continues to research and teach at Harvard. His laboratory remains one of the most productive and innovative **microbial genetics** labs in the world.

See also Bacterial adaptation; Microbial genetics

BEHRING, EMIL VON (1854-1917)

German bacteriologist

Emil von Behring's discovery of the **diphtheria** and **tetanus** antitoxins paved the way for the prevention of these diseases through the use of **immunization**. It also opened the door for the specific treatment of such diseases with the injection of immune serum. Behring's stature as a seminal figure in modern medicine and **immunology** was recognized in 1901, when he received the first Nobel Prize in physiology or medicine.

Emil Adolf von Behring was born in Hansdorf, West Prussia (now Germany). He was the eldest son of August Georg Behring, a schoolmaster with thirteen children, and his second wife, Augustine Zech Behring. Although his father planned for him to become a minister, young Behring had an inclination toward medicine. One of Behring's teachers, recognizing both the great promise and meager circumstances of his student, arranged for his admission to the Army Medical College in Berlin, where he was able to obtain a free medical education in exchange for future military service. Behring received his doctor of medicine degree in 1878, and two years later he passed the state examination that allowed him to practice medicine.

The army promptly sent Behring to Posen (now Poznan, Poland), then to Bonn in 1887, and finally back to Berlin in 1888. His first published papers, which date from this period, dealt with the use of iodoform as an antiseptic. After completing his military service in 1889 Behring became an assistant at the Institute of **Hygiene** in Berlin, joining a team of researchers

headed by German scientist **Robert Koch** (1843–1910), a leading light in the new science of bacteriology.

It was while working in Koch's laboratory that Behring began his pioneering investigations of diphtheria and tetanus. Both of these diseases are caused by **bacteria** that do not spread widely through the body, but produce generalized symptoms by excreting toxins. Diphtheria, nicknamed the "strangling angel" because of the way it obstructs breathing, was a terrible killer of children in the late nineteenth century. Its toxin had first been detected by others in 1888. Tetanus, likewise, was fatal more often than not. In 1889 the tetanus bacillus was cultivated in its pure state for the first time by the Japanese physician **Shibasaburo Kitasato** (1852–1931), another member of Koch's team.

The next year Behring and Kitasato jointly published their classic paper, "Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren" ("The Mechanism of Immunity in Animals to Diphtheria and Tetanus"). One week later Behring alone published another paper dealing with **immunity** against diphtheria and outlining five ways in which it could be achieved. These reports announced that injections of toxin from diphtheria or tetanus bacilli led animals to produce in their blood substances capable of neutralizing the disease poison.

Behring and Kitasato dubbed these substances antitoxins. Furthermore, injections of blood serum from an animal that had been given a chance to develop antitoxins to tetanus or diphtheria could confer immunity to the disease on other animals, and even cure animals that were already sick.

Several papers confirming and amplifying these results, including some by Behring himself, appeared in rapid succession. In 1893 Behring described a group of human diphtheria patients who were treated with antitoxin. That same year, he was given the title of professor. However, Behring's diphtheria antitoxin did not yield consistent results. It was the bacteriologist **Paul Ehrlich** (1854–1915), another of the talented associates in Koch's lab, who was chiefly responsible for standardizing the antitoxin, thus making it practical for widespread therapeutic use. Working together, Ehrlich and Behring also showed that high-quality antitoxin could be obtained from horses, as well as from the sheep used previously, opening the way for large-scale production of the antitoxin.

In 1894 Behring accepted a position as professor at the University of Halle. A year later he was named a professor and director of the Institute of Hygiene at the University of Marburg. Thereafter he focused much of his attention on the problem of immunization against **tuberculosis**. His assumption, unfounded as it turned out, was that different forms of the disease in humans and in cattle were closely related. He tried immunizing calves with a weakened strain of the human tuberculosis bacillus, but the results were disappointing. Although his bovine **vaccine** was widely used for a time in Germany, Russia, Sweden, and the United States, it was found that the cattle excreted dangerous **microorganisms** afterward. Nevertheless, Behring's basic idea of using a bacillus from one species to benefit another influenced the development of later vaccines.

Behring did not entirely abandon his work on diphtheria during this period. In 1913 he announced the development of a toxin-antitoxin mixture that resulted in longer-lasting immunity than did antitoxin serum alone. This approach was a forerunner of modern methods of preventing, rather than just treating, the disease. Today, children are routinely and effectively vaccinated against diphtheria and tetanus.

However, the first great drop in diphtheria mortality was due to the antitoxin therapy introduced earlier by Behring, and it is for this contribution that he is primarily remembered. The fall in the diphtheria death rate around the turn of the century was sharp. In Germany alone, an estimated 45,000 lives per year were saved. Accordingly, Behring received the 1901 Nobel Prize "for his work on serum therapy, especially its application against diphtheria, by which he... opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths." Behring was also elevated to the status of nobility and shared a sizable cash prize from the Paris Academy of Medicine with **Émile Roux**, the French bacteriologist who was one of the men who had discovered the diphtheria toxin in 1888. In addition, Behring was granted honorary memberships in societies in Italy, Turkey, France, Hungary, and Russia.

There were other financial rewards as well. From 1901 onward, ill health prevented Behring from giving regular lectures, so he devoted himself to research. A commercial firm in which he had a financial interest built a well-equipped laboratory for his use in Marburg, Germany. Then, in 1914, Behring established his own company to manufacture serums and vaccines. The profits from this venture allowed him to keep a large estate at Marburg, on which he grazed cattle used in experiments. This house was a gathering place of society. Behring also owned a vacation home on the island of Capri in the Mediterranean.

In 1896 Behring married the daughter of the director of a Berlin hospital. The couple had seven children. Despite outward appearances of personal and professional success, Behring was subject to frequent bouts of serious depression. He contracted **pneumonia** in 1917 and soon after died in Marburg, Germany.

See also Antibody and antigen; Antibody formation and kinetics; Bacteria and bacterial infection; History of immunology; History of microbiology; History of public health; Immune stimulation, as a vaccine; Immune system; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunization

Beijerinck, Martinus Willem (1851-1931)

Dutch botanist

Born in Amsterdam, Martinus Willem Beijerinck was the son of a tobacco dealer who went bankrupt. In response to his father's misfortune, Beijerinck would devote most of his scientific career to the **tobacco mosaic virus**, a pathogen causing

an economically devastating disease that dwarfs tobacco plants and mottles their leaves.

Beijerinck, who graduated from the Delft Polytechnic School, began his research under the assumption that the tobacco mosaic disease was caused by an unidentified bacterium or a parasite. Attempting to isolate the causative agent, Beijerinck filtered the sap of an infected plant to remove all known **bacteria**; however, the resulting liquid was still infective. In addition, the filtered substance was capable of infecting another plant, which could infect another, demonstrating that the substance had the ability to multiply and grow. The Russian botanist Dmitri Ivanovsky had come up against the same type of agent, but had failed to report its existence, assuming instead that his research was flawed.

In 1898 Beijerinck published his work, which maintained that tobacco mosaic disease was caused not by bacteria, but by a living liquid virus that infected only growing plant organs where cellular division allowed it to multiply. This new agent he called a filterable virus, from Latin meaning filterable poison. **Louis Pasteur** had speculated about the existence of germs that were smaller than bacteria, but did not conduct research into this phenomenon. Beijerinck asserted that the virus was liquid, but this theory was later disproved by Wendell Stanley, who demonstrated the particulate nature of **viruses**. Beijerinck, nevertheless, set the stage for twentieth-century virologists to uncover the secrets of viral pathogens now known to cause a wide range of plant and animal (including human) diseases.

See also Virology; Virus replication; Viruses and responses to viral infection

BERG, PAUL (1926-)

American biochemist

Paul Berg developed a technique for splicing together (DNA)—the substance that carries genetic information in living cells from generation to generation—from different types of organisms. Berg's achievement, one of the most fundamental technical contributions to the field of genetics in the twentieth century, gave scientists an invaluable tool for studying the structure of viral **chromosomes** and the biochemical basis of human genetic diseases. It also allowed researchers to turn simple organisms into chemical factories that churn out valuable medical drugs. In 1980 Berg was awarded the Nobel Prize in chemistry for pioneering this procedure, now referred to as recombinant **DNA** technology.

The commercial application of Berg's work underlies a large and growing industry dedicated to manufacturing drugs and other chemicals. Moreover, the ability to recombine pieces of DNA and transfer them into cells is the basis of an important new medical approach to treating diseases by a technique called **gene** therapy.

Berg was born in Brooklyn, New York, one of three sons of Harry Berg, a clothing manufacturer, and Sarah Brodsky, a homemaker. He attended public schools, including Abraham Lincoln High School, from which he graduated in 1943. In a 1980 interview reported in the *New York Times,* Berg credited a "Mrs. Wolf," the woman who ran a science club after school, with inspiring him to become a researcher. He graduated from high school with a keen interest in microbiology and entered Pennsylvania State University, where he received a degree in **biochemistry** in 1948.

Before entering graduate school, Berg served in the United States Navy from 1943 to 1946. On September 13, 1947, he married Mildred Levy; the couple later had one son. After completing his duty in the navy, Berg continued his study of biochemistry at Western Reserve University (now Case Western Reserve University) in Cleveland, Ohio, where he was a National Institutes of Health fellow from 1950 to 1952 and received his doctorate degree in 1952. He did postdoctoral training as an American Cancer Society research fellow, working with Herman Kalckar at the Institute of Cytophysiology in Copenhagen, Denmark, from 1952 to 1953. From 1953 to 1954 he worked with biochemist Arthur Kornberg at Washington University in St. Louis, Missouri, and held the position of scholar in cancer research from 1954 to 1957.

He became an assistant professor of microbiology at the University of Washington School of Medicine in 1956, where he taught and did research until 1959. Berg left St. Louis that year to accept the position of professor of biochemistry at Stanford University School of Medicine. Berg's background in biochemistry and microbiology shaped his research interests during graduate school and beyond, steering him first into studies of the molecular mechanisms underlying intracellular **protein synthesis**.

During the 1950s, Berg tackled the problem of how amino acids, the building blocks of proteins, are linked together according to the template carried by a form of **RNA** (**ribonucleic acid**, the "decoded" form of DNA) called messenger RNA (mRNA). A current theory, unknown to Berg at the time, held that the amino acids did not directly interact with RNA but were linked together in a chain by special molecules called joiners, or adapters. In 1956 Berg demonstrated just such a molecule, which was specific to the amino acid methionine. Each amino acid has its own such joiners, which are now called transfer RNA (tRNA).

This discovery helped to stoke Berg's interest in the structure and function of genes, and fueled his ambition to combine genetic material from different species in order to study how these individual units of heredity worked. Berg reasoned that by recombining a gene from one species with the genes of another, he would be able to isolate and study the transferred gene in the absence of confounding interactions with its natural, neighboring genes in the original organism.

In the late 1960s, while at Stanford, Berg began studying genes of the monkey tumor virus SV40 as a model for understanding how mammalian genes work. By the 1970s, he had mapped out where on the DNA the various viral genes occurred, identified the specific sequences of nucleotides in the genes, and discovered how the SV40 genes affect the DNA of host organisms they infect. It was this work with SV40 genes that led directly to the development of recombinant DNA technology. While studying how genes controlled the production of specific proteins, Berg also was trying to under-

stand how normal cells seemed spontaneously to become cancerous. He hypothesized that cells turned cancerous because of some unknown interaction between genes and cellular biochemistry.

In order to study these issues, Berg decided to combine the DNA of SV40, which was known to cause cancer in some animals, into the common intestinal bacterium ***Escherichia coli***. He thought it might be possible to smuggle the SV40 DNA into the bacterium by inserting it into the DNA of a type of virus, called a **bacteriophage**, that naturally infects *E. coli*.

A DNA molecule is composed of subunits called nucleotides, each containing a sugar, a phosphate group, and one of four nitrogenous bases. Structurally, DNA resembles a twisted ladder, or helix. Two long chains of alternating sugar and phosphate groups twist about each other, forming the sides of the ladder. A base attaches to each sugar, and hydrogen bonding between the bases—the rungs of the ladder—connects the two strands. The order or sequence of the bases determines the **genetic code**; and because bases match up in a complementary way, the sequence on one strand determines the sequence on the other.

Berg began his experiment by cutting the SV40 DNA into pieces using so-called **restriction enzymes**, which had been discovered several years before by other researchers. These **enzymes** let him choose the exact sites to cut each strand of the double helix. Then, using another type of enzyme called terminal transferase, he added one base at a time to one side of the double-stranded molecule. Thus, he formed a chain that extended out from the double-stranded portion. Berg performed the same biochemical operation on the phage DNA, except he changed the sequence of bases in the reconstructed phage DNA so it would be complementary to—and therefore readily bind to—the reconstructed SV40 section of DNA extending from the double-stranded portion. Such complementary extended portions of DNA that bind to each other to make recombinant DNA molecules are called "sticky ends."

This new and powerful technique offered the means to put genes into rapidly multiplying cells, such as **bacteria**, which would then use the genes to make the corresponding protein. In effect, scientists would be able to make enormous amounts of particular genes they wanted to study, or use simple organisms like bacteria to grow large amounts of valuable substances like human growth hormone, **antibiotics**, and insulin. Researchers also recognized that genetic engineering, as the technique was quickly dubbed, could be used to alter soil bacteria to give them the ability to "fix" nitrogen from the air, thus reducing the need for artificial fertilizers.

Berg had planned to inject the monkey virus SV40-bacteriophage DNA hybrid molecule into *E. coli*. But he realized the potential danger of inserting a mammalian tumor gene into a bacterium that exists universally in the environment. Should the bacterium acquire and spread to other *E. coli* dangerous, pathogenic characteristics that threatened humans or other species, the results might be catastrophic. In his own case, he feared that adding the tumor-causing SV40 DNA into such a common bacterium would be equivalent to planting a ticking cancer time bomb in humans who might subsequently become infected by altered bacteria that escaped from the lab. Rather than continue his ground-breaking experiment, Berg voluntarily halted his work at this point, concerned that the tools of genetic engineering might be leading researchers to perform extremely dangerous experiments.

In addition to this unusual voluntary deferral of his own research, Berg led a group of ten of his colleagues from around the country in composing and signing a letter explaining their collective concerns. Published in the July 26, 1974, issue of the journal *Science,* the letter became known as the "Berg letter." It listed a series of recommendations supported by the Committee on Recombinant DNA Molecules Assembly of Life Sciences (of which Berg was chairman) of the National Academy of Sciences.

The Berg letter warned, "There is serious concern that some of these artificial recombinant DNA molecules could prove biologically hazardous." It cited as an example the fact that *E. coli* can exchange genetic material with other types of bacteria, some of which cause disease in humans. "Thus, new DNA elements introduced into *E. coli* might possibly become widely disseminated among human, bacterial, plant, or animal populations with unpredictable effects." The letter also noted certain recombinant DNA experiments that should not be conducted, such as recombining genes for **antibiotic resistance** or bacterial toxins into bacterial strains that did not at present carry them; linking all or segments of DNA from cancer-causing or other animal **viruses** into **plasmids** or other viral DNAs that could spread the DNA to other bacteria, animals or humans, "and thus possibly increase the incidence of cancer or other disease."

The letter also called for an international meeting of scientists from around the world "to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules." That meeting was held in Pacific Grove, California, on February 27, 1975, at **Asilomar** and brought together a hundred scientists from sixteen countries. For four days, Berg and his fellow scientists struggled to find a way to safely balance the potential hazards and inestimable benefits of the emerging field of genetic engineering. They agreed to collaborate on developing safeguards to prevent genetically engineered organisms designed only for laboratory study from being able to survive in humans. And they drew up professional standards to govern research in the new technology, which, though backed only by the force of moral persuasion, represented the convictions of many of the leading scientists in the field. These standards served as a blueprint for subsequent federal regulations, which were first published by the National Institutes of Health in June 1976. Today, many of the original regulations have been relaxed or eliminated, except in the cases of recombinant organisms that include extensive DNA regions from very pathogenic organisms. Berg continues to study genetic recombinants in mammalian cells and gene therapy. He is also doing research in **molecular biology** of HIV–1.

The Nobel Award announcement by the Royal Swedish Academy of Sciences cited Berg "for his fundamental studies of the biochemistry of nucleic acids with particular regard to recombinant DNA." Berg's legacy also includes his principled actions in the name of responsible scientific inquiry.

Berg was named the Sam, Lula, and Jack Willson Professor of Biochemistry at Stanford in 1970, and was chairman of the Department of Biochemistry there from 1969 to 1974. He was also director of the Beckman Center for Molecular and Genetic Medicine (1985), senior postdoctoral fellow of the National Science Foundation (1961–68), and nonresident fellow of the Salk Institute (1973–83). He was elected to the advisory board of the Jane Coffin Childs Foundation of Medical Research, serving from 1970 to 1980. Other appointments include the chair of the scientific advisory committee of the Whitehead Institute (1984–90) and of the national advisory committee of the Human Genome Project (1990). He was editor of *Biochemistry and Biophysical Research Communications* (1959–68), and a trustee of Rockefeller University (1990–92). He is a member of the international advisory board, Basel Institute of **Immunology**.

Berg received many awards in addition to the Nobel Prize, among them the American Chemical Society's Eli Lilly Prize in biochemistry (1959); the V. D. Mattia Award of the Roche Institute of Molecular Biology (1972); the Albert Lasker Basic Medical Research Award (1980); and the National Medal of Science (1983). He is a fellow of the American Academy of Arts and Sciences, and a foreign member of the Japanese Biochemistry Society and the Académie des Sciences, France.

See also Asilomar conferences; Bacteriophage and bacteriophage typing; Immunodeficiency disease syndromes; Immunogenetics

Bergey, David Hendricks (1860-1937)

American bacteriologist

David Hendricks Bergey was an American bacteriologist. He was the primary author of *Bergey's Manual of Determinative Bacteriology*, which has been a fundamentally important reference book for the identification and classification of **bacteria** since its publication in 1923.

Bergey was born in the state of Pennsylvania where he remained his entire life. In his early years, Bergey was a schoolteacher he taught in schools of Montgomery Country.

He left this occupation to attend the University of Pennsylvania. In 1884 he receive both a B.S. and M.D. degrees. From then until 1893 he was a practicing physician. In 1893 he became a faculty member at his alma mater. The following year he was appointed the Thomas A. Scott fellow in the Laboratory of **Hygiene**.

In 1916, he received a doctor of **public health** degree. His career at the university flourished. He was professor of hygiene and bacteriology in the undergraduate and graduate schools, and became director of the Laboratory of Hygiene in 1929. He served as director and had other university appointments from 1929 until his retirement in 1932.

From 1932 until his death in 1937 he was director of biological research at the National Drug Company in Philadelphia.

During his years at the University of Pennsylvania, Bergey was a prolific and varied researcher. His research included **tuberculosis**, food preservatives, the engulfment of particles and foreign organisms by immune cells (a phenomenon termed **phagocytosis**), and the enhanced immune reaction of an organism to an antigenic target (called **anaphylaxis**). He was also responsible for determining the interrelations and differences that helped identify the organisms in a class called Schizomycetes.

This latter research activity also formed the basis for his most well known accomplishment. In the early years of the twentieth century Bergey became chair of an organizational committee whose mandate was to devise a classification scheme for all known bacteria, a scheme that could be used to identify unknown bacteria based on various criteria (such as Gram stain reaction, shape, appearance of colonies, and on a variety of biochemical reactions). In 1923, he and four other bacteriologists published the first edition of *Bergey's Manual of Determinative Bacteriology*.

The first three editions of the *Manual* were published by the Society of American Bacteriologists (now called the American Society for Microbiology). During the preparation of the fourth edition in 1934 it became apparent that the financial constraints of the Society were making publication of the *Manual* difficult. Subsequently, it was agreed by the Society and Bergey that he would assume all rights, title and interest in the *Manual*. In turn, an educational trust was created to oversee and fund the publication of future editions of the *Manual*. The Bergey's Trust continues to the present day.

From the first edition to the present day, the Bergey's manual has continued to be updated and new revisions published every few years. In addition to the *Manual*, Bergey published the *Handbook of Practical Hygiene* in 1899 and *The Principles of Hygiene* in 1901.

See also History of public health

Berkeley, Reverend M. J. (1803-1889)

British cleric and fungal researcher and classifier

M.J. Berkeley lived in Britain during the nineteenth century. An ordained minister, he is best known for his contributions to the study and classification of **fungi**. He compiled a number of volumes of literature on fungi. One of the best-known examples is the massive and well-illustrated *Outlines of British Fungology*, which was published in 1860. In this volume, Berkeley detailed a thousand species of fungi then known to be native to the British Isles. He was involved active in chronicling the discoveries of others. As examples, he co-authored a paper that described the findings of a United States–Japan expedition that found many species of fungi in the North Pacific in 1852–1853, and wrote several treatises on botanic expeditions to New Zealand and Antarctica.

Another of Berkeley's important contributions were connected to the Irish potato famine. From 1846 to 1851, the

loss of the potato crops in Ireland resulted in the death due to starvation of at least one million people, and the mass emigration of people to countries including the United States and Canada. The famine was attributed to many sources, many of which had no basis in scientific reason. Dr. C. Montane, a physician in the army of Napoleon, first described the presence of fungus on potatoes after a prolonged period of rain. He shared this information with Berkeley, who surmised that the fungus was the cause of the disease. Berkeley was alone in this view. Indeed, Dr. John Lindley, a botany professor at University College in London, and a professional rival of Berkeley's, hotly and publicly disputed the idea. Lindley blamed the famine on the damp weather of Ireland. Their differing opinions were published in *The Gardener's Chronicles*.

With time, Berkeley's view was proven to be correct. A committee formed to arbitrate the debate sided with Berkeley. On the basis of the decision, farmers were advised to store their crop in well-ventilated pits, which aided against fungal growth.

The discovery that the fungus *Phytophthora infestans* was the basis of the potato blight represented the first disease known to be caused by a microorganism, and marked the beginning of the scientific discipline of plant pathology.

Berkeley also contributed to the battle against poultry mildew, a fungal disease that produced rotting of vines. The disease could e devastating. For example, the appearance of poultry mildew in Madeira in the 1850s destroyed the local wine-based economy, which led to widespread starvation and emigration. Berkeley was one of those who helped established the cause of the infestation.

BEVERIDGE, TERRANCE J. (1945-)

Canadian microbiologist

Terrance (Terry) J. Beveridge has fundamentally contributed to the understanding of the structure and function of **bacteria**.

Beveridge was born in Toronto, Ontario, Canada. His early schooling was also in that city. He graduated with a B.Sc. from the University of Toronto in 1968, a Dip. Bact. in 1969, and an M.Sc. in oral microbiology in 1970. Intending to become a dentist, he was drawn to biological research instead. This interest led him to the University of Western Ontario laboratory of Dr. **Robert Murray**, where he completed his Ph.D. dissertation in 1974.

His Ph.D. research focused on the use of various techniques to probe the structure of bacteria. In particular, he developed an expertise in electron microscopy. His research interest in the molecular structure of bacteria was carried on in his appointment as an Assistant Professor at the University of Guelph in 1975. He became an Associate Professor in 1983 and a tenured Professor in 1986. He has remained at the University of Guelph to the present day.

Beveridge's interest in **bacterial ultrastructure** had led to many achievements. He and his numerous students and research colleagues pioneered the study of the binding of metals by bacteria, and showed how these metals function to cement components of the cell wall of Gram-negative and Gram-positive bacteria together. Bacteria were shown to be capable of precipitating metals from solution, producing what he termed microfossils. Indeed, Beveridge and others have discovered similar appearing microfossils in rock that is millions of years old. Such bacteria are now thought to have played a major role in the development of conditions suitable for the explosive diversity of life on Earth.

In 1981, Beveridge became Director of a Guelph-based electron microscopy research facility. Using techniques including scanning tunneling microscopy, atomic force microscopy and confocal microscopy, the molecular nature of regularly-structured protein layers on a number of bacterial species have been detailed. Knowledge of the structure is allowing strategies to overcome the layer's role as a barrier to antibacterial compounds. In another accomplishment, the design and use of metallic probes allowed Beveridge to deduce the actual mechanism of operation of the Gram stain. The mechanism of the stain technique, of bedrock importance to microbiology, had not been known since the development of the stain in the nineteenth century.

In the 1980s, in collaboration with Richard Blakemore's laboratory, used electron microscopy to reveal the structure, arrangement and growth of the magnetically-responsive particles in *Aquaspirillum magnetotacticum*. In the past decade, Beveridge has discovered how bacterial life manages to survive in a habitat devoid of oxygen, located in the Earth's crust miles beneath the surface. These discoveries have broadened human knowledge of the diversity of life on the planet.

Another accomplishment of note has been the finding that portions of the bacterial cell wall that are spontaneously released can be used to package **antibiotics** and deliver them to the bacteria. This novel means of killing bacteria shows great potential in the treatment of bacterial infections.

These and other accomplishment have earned Beveridge numerous awards. In particular, he received the Steacie Award in 1984, an award given in recognition of outstanding fundamental research by a researcher in Canada, and the Culling Medal from the National Society of Histotechnology in 2001.

See also Bacterial ultrastructure; Electron microscope examination of microorganisms; Magnetotactic bacteria

BIOCHEMICAL ANALYSIS TECHNIQUES

Biochemical analysis techniques refer to a set of methods, assays, and procedures that enable scientists to analyze the substances found in living organisms and the chemical reactions underlying life processes. The most sophisticated of these techniques are reserved for specialty research and diagnostic laboratories, although simplified sets of these techniques are used in such common events as testing for illegal drug abuse in competitive athletic events and monitoring of blood sugar by diabetic patients.

To perform a comprehensive biochemical analysis of a biomolecule in a biological process or system, the biochemist

Technician performing biochemical analysis.

typically needs to design a strategy to detect that biomolecule, isolate it in pure form from among thousands of molecules that can be found in an extracts from a biological sample, characterize it, and analyze its function. An assay, the biochemical test that characterizes a molecule, whether quantitative or semi-quantitative, is important to determine the presence and quantity of a biomolecule at each step of the study. Detection assays may range from the simple type of assays provided by spectrophotometric measurements and gel staining to determine the concentration and purity of proteins and nucleic acids, to long and tedious bioassays that may take days to perform.

The description and characterization of the molecular components of the cell succeeded in successive stages, each one related to the introduction of new technical tools adapted to the particular properties of the studied molecules. The first studied biomolecules were the small building blocks of larger and more complex macromolecules, the amino acids of proteins, the bases of nucleic acids and sugar monomers of complex carbohydrates. The molecular characterization of these elementary components was carried out thanks to techniques used in organic chemistry and developed as early as the nineteenth century. Analysis and characterization of complex macromolecules proved more difficult, and the fundamental techniques in protein and nucleic acid and protein purification and sequencing were only established in the last four decades.

Most biomolecules occur in minute amounts in the cell, and their detection and analysis require the biochemist to first assume the major task of purifying them from any **contamination**. Purification procedures published in the specialist literature are almost as diverse as the diversity of biomolecules and are usually written in sufficient details that they can be reproduced in different laboratory with similar results. These procedures and protocols, which are reminiscent of recipes in cookbooks have had major influence on the progress of biomedical sciences and were very highly rated in scientific literature.

The methods available for purification of biomolecules range from simple precipitation, centrifugation, and gel **electrophoresis** to sophisticated chromatographic and affinity techniques that are constantly undergoing development and improvement. These diverse but interrelated methods are based on such properties as size and shape, net charge and bioproperties of the biomolecules studied.

Centrifugation procedures impose, through rapid spinning, high centrifugal forces on biomolecules in solution, and cause their separations based on differences in weight. Electrophoresis techniques take advantage of both the size and charge of biomolecules and refer to the process where biomolecules are separated because they adopt different rates of migration toward positively (anode) or negatively (cathode) charged poles of an electric field. Gel electrophoresis methods are important steps in many separation and analysis techniques in the studies of **DNA**, proteins and lipids. Both western blotting techniques for the assay of proteins and southern and northern analysis of DNA rely on gel electrophoresis. The completion of DNA sequencing at the different human genome centers is also dependent on gel electrophoresis. A powerful modification of gel electrophoresis called two-dimensional gel electrophoresis is predicted to play a very important role in the accomplishment of the proteome projects that have started in many laboratories.

Chromatography techniques are sensitive and effective in separating and concentrating minute components of a mixture and are widely used for quantitative and qualitative analysis in medicine, industrial processes, and other fields. The method consists of allowing a liquid or gaseous solution of the test mixture to flow through a tube or column packed with a finely divided solid material that may be coated with an active chemical group or an adsorbent liquid. The different components of the mixture separate because they travel through the tube at different rates, depending on the interactions with the porous stationary material. Various chromatographic separation strategies could be designed by modifying the chemical components and shape of the solid adsorbent material. Some chromatographic columns used in gel chromatography are packed with porous stationary material, such that the small molecules flowing through the column diffuse into the matrix and will be delayed, whereas larger molecules flow through the column more quickly. Along with ultracentrifugation and

gel electrophoresis, this is one of the methods used to determine the molecular weight of biomolecules. If the stationary material is charged, the chromatography column will allow separation of biomolecules according to their charge, a process known as ion exchange chromatography. This process provides the highest resolution in the purification of native biomolecules and is valuable when both the purity and the activity of a molecule are of importance, as is the case in the preparation of all **enzymes** used in **molecular biology**. The biological activity of biomolecules has itself been exploited to design a powerful separation method known as affinity chromatography. Most biomolecules of interest bind specifically and tightly to natural biological partners called ligands: enzymes bind substrates and cofactors, hormones bind receptors, and specific **immunoglobulins** called antibodies can be made by the **immune system** that would in principle interact with any possible chemical component large enough to have a specific conformation. The solid material in an affinity chromatography column is coated with the ligand and only the biomolecule that specifically interact with this ligand will be retained while the rest of a mixture is washed away by excess solvent running through the column.

Once a pure biomolecule is obtained, it may be employed for a specific purpose such as an enzymatic reaction, used as a therapeutic agent, or in an industrial process. However, it is normal in a research laboratory that the biomolecule isolated is novel, isolated for the first time and, therefore, warrants full characterization in terms of structure and function. This is the most difficult part in a biochemical analysis of a novel biomolecule or a biochemical process, usually takes years to accomplish, and involves the collaboration of many research laboratories from different parts of the world.

Recent progress in biochemical analysis techniques has been dependant upon contributions from both chemistry and biology, especially **molecular genetics** and molecular biology, as well as engineering and information technology. Tagging of proteins and nucleic acids with chemicals, especially **fluorescent dyes**, has been crucial in helping to accomplish the sequencing of the human genome and other organisms, as well as the analysis of proteins by chromatography and mass spectrometry. Biochemical research is undergoing a change in paradigm from analysis of the role of one or a few molecules at a time, to an approach aiming at the characterization and functional studies of many or even all biomolecules constituting a cell and eventually organs. One of the major challenges of the post-genome era is to assign functions to all of the **gene** products discovered through the genome and cDNA sequencing efforts. The need for functional analysis of proteins has become especially eminent, and this has led to the renovated interest and major technical improvements in some protein separation and analysis techniques. Two-dimensional gel electrophoresis, high performance liquid and capillary chromatography as well as mass spectrometry are proving very effective in separation and analysis of abundant change in highly expressed proteins. The newly developed hardware and software, and the use of automated systems that allow analysis of a huge number of samples simultaneously, is making it possible to analyze a large number of proteins in a shorter time and with higher accuracy. These approaches are making it possible to study global protein expression in cells and tissues, and will allow comparison of protein products from cells under varying conditions like differentiation and activation by various stimuli such as stress, hormones, or drugs. A more specific assay to analyze protein function *in vivo* is to use expression systems designed to detect protein-protein and DNA-protein interactions such as the **yeast** and bacterial hybrid systems. Ligand-receptor interactions are also being studied by novel techniques using biosensors that are much faster than the conventional immunochemical and colorimetric analyzes.

The combination of large scale and automated analysis techniques, bioinformatic tools, and the power of genetic manipulations will enable scientists to eventually analyze processes of cell function to all depths.

See also Bioinformatics and computational biology; Biotechnology; Fluorescence *in situ* hybridization; Immunological analysis techniques; Luminescent bacteria

BIOCHEMISTRY

Biochemistry seeks to describe the structure, organization, and functions of living matter in molecular terms. Essentially two factors have contributed to the excitement in the field today and have enhanced the impact of research and advances in biochemistry on other life sciences. First, it is now generally accepted that the physical elements of living matter obey the same fundamental laws that govern all matter, both living and non-living. Therefore the full potential of modern chemical and physical theory can be brought in to solve certain biological problems. Secondly, incredibly powerful new research techniques, notably those developing from the fields of biophysics and **molecular biology**, are permitting scientists to ask questions about the basic process of life that could not have been imagined even a few years ago.

Biochemistry now lies at the heart of a revolution in the biological sciences and it is nowhere better illustrated than in the remarkable number of Nobel Prizes in Chemistry or Medicine and Physiology that have been won by biochemists in recent years. A typical example is the award of the 1988 Nobel Prize for Medicine and Physiology, to **Gertrude Elion** and George Hitchings of the United States and Sir James Black of Great Britain for their leadership in inventing new drugs. Elion and Hitchings developed chemical analogs of nucleic acids and vitamins which are now being used to treat leukemia, bacterial infections, **malaria**, gout, herpes virus infections and **AIDS**. Black developed beta-blockers that are now used to reduce the risk of heart attack and to treat diseases such as asthma. These drugs were designed and not discovered through random organic synthesis. Developments in knowledge within certain key areas of biochemistry, such as protein structure and function, nucleic acid synthesis, enzyme mechanisms, receptors and metabolic control, vitamins, and coenzymes all contributed to enable such progress to be made.

Two more recent Nobel Prizes give further evidence for the breadth of the impact of biochemistry. In 1997, the

Chemistry Prize was shared by three scientists: the American Paul Boyer and the British J. Walker for their discovery of the "rotary engine" that generates the energy-carrying compound ATP, and the Danish J. Skou, for his studies of the "pump" that drives sodium and potassium across membranes. In the same year, the Prize in Medicine and Physiology went to **Stanley Prusiner**, for his studies on the prion, the agent thought to be responsible for "mad cow disease" and several similar human conditions.

Biochemistry draws on its major themes from many disciplines. For example from organic chemistry, which describes the properties of biomolecules; from biophysics, which applies the techniques of physics to study the structures of biomolecules; from medical research, which increasingly seeks to understand disease states in molecular terms and also from nutrition, microbiology, physiology, cell biology and genetics. Biochemistry draws strength from all of these disciplines but is also a distinct discipline, with its own identity. It is distinctive in its emphasis on the structures and relations of biomolecules, particularly **enzymes** and biological catalysis, also on the elucidation of metabolic pathways and their control and on the principle that life processes can, at least on the physical level, be understood through the laws of chemistry. It has its origins as a distinct field of study in the early nineteenth century, with the pioneering work of Freidrich Wöhler. Prior to Wöhler's time it was believed that the substance of living matter was somehow quantitatively different from that of nonliving matter and did not behave according to the known laws of physics and chemistry. In 1828 Wöhler showed that urea, a substance of biological origin excreted by humans and many animals as a product of nitrogen **metabolism**, could be synthesized in the laboratory from the inorganic compound ammonium cyanate. As Wöhler phrased it in a letter to a colleague, "I must tell you that I can prepare urea without requiring a kidney or an animal, either man or dog." This was a shocking statement at the time, for it breached the presumed barrier between the living and the nonliving. Later, in 1897, two German brothers, Eduard and Hans Buchner, found that extracts from broken and thoroughly dead cells from **yeast**, could nevertheless carry out the entire process of **fermentation** of sugar into ethanol. This discovery opened the door to analysis of biochemical reactions and processes in vitro (Latin "in glass"), meaning in the test tube rather than in vivo, in living matter. In succeeding decades many other metabolic reactions and reaction pathways were reproduced in vitro, allowing identification of reactants and products and of enzymes, or biological catalysts, that promoted each biochemical reaction.

Until 1926, the structures of enzymes (or "ferments") were thought to be far too complex to be described in chemical terms. But in 1926, J.B. Sumner showed that the protein urease, an enzyme from jack beans, could be crystallized like other organic compounds. Although proteins have large and complex structures, they are also organic compounds and their physical structures can be determined by chemical methods.

Today, the study of biochemistry can be broadly divided into three principal areas: (1) the structural chemistry of the components of living matter and the relationships of biological function to chemical structure; (2) metabolism, the totality of chemical reactions that occur in living matter; and (3) the chemistry of processes and substances that store and transmit biological information. The third area is also the province of **molecular genetics**, a field that seeks to understand heredity and the expression of genetic information in molecular terms.

Biochemistry is having a profound influence in the field of medicine. The molecular mechanisms of many diseases, such as sickle cell anemia and numerous errors of metabolism, have been elucidated. Assays of enzyme activity are today indispensable in clinical diagnosis. To cite just one example, liver disease is now routinely diagnosed and monitored by measurements of blood levels of enzymes called transaminases and of a hemoglobin breakdown product called bilirubin. **DNA** probes are coming into play in diagnosis of genetic disorders, infectious diseases and cancers. Genetically engineered strains of **bacteria** containing recombinant DNA are producing valuable proteins such as insulin and growth hormone. Furthermore, biochemistry is a basis for the rational design of new drugs. Also the rapid development of powerful biochemical concepts and techniques in recent years has enabled investigators to tackle some of the most challenging and fundamental problems in medicine and physiology. For example in embryology, the mechanisms by which the fertilized embryo gives rise to cells as different as muscle, brain and liver are being intensively investigated. Also, in anatomy, the question of how cells find each other in order to form a complex organ, such as the liver or brain, are being tackled in biochemical terms. The impact of biochemistry is being felt in many areas of human life through this kind of research, and the discoveries are fuelling the growth of the life sciences as a whole.

See also Antibody-antigen, biochemical and molecular reactions; Biochemical analysis techniques; Biogeochemical cycles; Bioremediation; Biotechnology; Immunochemistry; Immunological analysis techniques; Miller-Urey experiment; Nitrogen cycle in microorganisms; Photosynthesis

BIODEGRADABLE SUBSTANCES

The increase in public environmental awareness and the recognition of the urgent need to control and reduce pollution are leading factors in the recent augment of scientific research for new biodegradable compounds. Biodegradable compounds could replace others that harm the environment and pose hazards to **public health**, and animal and plant survival. Biodegradation, i.e., the metabolization of substances by **bacteria**, **yeast**, **fungi**, from which these organisms obtain nutrients and energy, is an important natural resource for the development of new environmental-friendly technologies with immediate impact in the chemical industry and other economic activities. Research efforts in this field are two-fold: to identify and/or develop transgenic biological agents that digest specific existing compounds in polluted soils and water,

and to develop new biodegradable compounds to replace hazardous chemicals in industrial activity. Research is, therefore, aimed at **bioremediation**, which could identify biological agents that rapidly degrade existing pollutants in the environment, such as heavy metals and toxic chemicals in soil and water, explosive residues, or spilled petroleum. Crude oil however, is naturally biodegradable, and species of hydrocarbon-degrading bacteria are responsible for an important reduction of petroleum levels in reservoirs, especially at temperatures below 176° F (80° C). The **selection**, **culture**, and even genetic manipulation of some of these species may lead to a bioremediation technology that could rapidly degrade oil accidentally spilled in water.

The search for a biodegradable substitute for plastic polymers, for instance, is of high environmental relevance, since plastic waste (bags, toys, plastic films, packing material, etc.) is a major problem in garbage disposal and its recycling process is not pollution-free. In the 1980s, research of polyhydroxybutyrate, a biodegradable thermoplastic derived from bacterial **metabolism** was started and then stalled due to the high costs involved in **fermentation** and extraction. Starch is another trend of research in the endeavor to solve this problem, and starch-foamed packing material is currently in use in many countries, as well as molded starch golf tees. However, physical and chemical properties of starch polymers have so far prevented its use for other industrial purposes in replacement of plastic. Some scientists suggest that polyhydroxybutyrate research should now be increased to benefit from new biotechnologies, such as the development of transgenic corn, with has the ability to synthesize great amounts of the compound. This corn may one day provide a cost-effective biodegradable raw material to a new biodegradable plastics industry.

Another field for biodegradable substances usage is the pharmaceutical industry, where biomedical research focuses on non-toxic polymers with physicochemical thermo-sensitivity as a matrix for drug delivering. One research group at the University of Utah at Salt Lake City in 1997, for instance, synthesized an injectable polymer that forms a non-toxic biodegradable hydro gel that acts as a sustained-release matrix for drugs.

Transgenic plants expressing microbial genes whose products are degradative **enzymes** may constitute a potential solution in the removal of explosive residues from water and soils. A group of University of Cambridge and University of Edinburgh scientists in the United Kingdom developed transgenic tobacco plants that express an enzyme (pentaerythritol tetranitrate reductase) that degrades nitrate ester and nitro aromatic explosive residues in contaminated soils.

Another environmental problem is the huge amounts of highly stable and non-biodegradable hydrocarbon compounds that are discarded in landfills, and are known as polyacrylates. Polyacrylates are utilized as absorbent gels in disposable diapers, and feminine **hygiene** absorbents, as well as added to detergents as dispersants, and are discharged through sewage into underwater sheets, rivers, and lakes. A biodegradable substitute, however, known as polyaspartate, already exists, and is presently utilized in farming and oil drilling. Polyaspartate polymers are degradable by bacteria because the molecular backbone is constituted by chains of amino acids; whereas polyacrylates have backbones made of hydrocarbon compounds.

The main challenge in the adoption of biodegradable substances as a replacement for existing hazardous chemicals and technologies is cost effectiveness. Only large-scale production of environmental friendly compounds can decrease costs. Public education and consumer awareness may be a crucial factor in the progress and consolidation of "green" technologies in the near future.

See also Amino acid chemistry; Biotechnology; Economic uses and benefits of microorganisms; Transgenics; Waste water treatment

BIOFILM FORMATION AND DYNAMIC BEHAVIOR

Biofilms are populations of **microorganisms** that form following the adhesion of **bacteria**, algae, **yeast**, or **fungi** to a surface. These surface growths can be found in natural settings, such as on rocks in streams, and in infections, such as on catheters. Both living and inert surfaces, natural and artificial, can be colonized by microorganisms.

Up until the 1980s, the biofilm mode of growth was regarded as more of a scientific curiosity than an area for serious study. Then, evidence accumulated to demonstrate that biofilm formation is the preferred mode of growth for microbes. Virtually every surface that is in contact with microorganisms has been found to be capable of sustaining biofilm formation.

The best-studied biofilms are those formed by bacteria. Much of the current knowledge of bacterial biofilm comes from laboratory studies of pure cultures of bacteria. However, biofilm can also be comprised of a variety of bacteria. Dental **plaque** is a good example. Many species of bacteria can be present in the exceedingly complex biofilm that form on the surface of the teeth and gums.

The formation of a biofilm begins with a clean, bacteria-free surface. Bacteria that are growing in solution (**planktonic bacteria**) encounter the surface. Attachment to the surface can occur specifically, via the recognition of a surface receptor by a component of the bacterial surface, or non-specifically. The attachment can be mediated by **bacterial appendages**, such as flagella, cilia, or the holdfast of *Caulobacter crescentus*.

If the attachment is not transient, the bacterium can undergo a change in its character. Genes are stimulated to become expressed by some as yet unclear aspect of the surface association. This process is referred to as auto-induction. A common manifestation of the genetic change is the production and excretion of a large amount of a sugary material. This material covers the bacterium and, as more bacteria accumulate from the fluid layer and from division of the surface-adherent bacteria, the entire mass can become buried in

the sugary network. This mass represents the biofilm. The sugar constituent is known as **glycocalyx**, exopolysaccharide, or slime.

As the biofilm thickens and multiple layers of bacteria build up, the behavior of the bacteria becomes even more complex. Studies using instruments such as the confocal **microscope** combined with specific fluorescent probes of various bacterial structures and functional activities have demonstrated that the bacteria located deeper in the biofilm cease production of the slime and adopt an almost dormant state. In contrast, bacteria at the biofilm's periphery are faster-growing and still produce large quantities of the slime. These activities are coordinated. The bacteria can communicate with one another by virtue of released chemical compounds. This so-called **quorum sensing** enables a biofilm to grow and sense when bacteria should be released so as to colonize more distant surfaces.

The technique of confocal microscopy allows biofilms to be examined without disrupting them. Prior to the use of the technique, biofilms were regarded as being a homogeneous distribution of bacteria. Now it is known that this view is incorrect. In fact, bacteria are clustered together in "microcolonies" inside the biofilm, with surrounding regions of bacteria-free slime or even channels of water snaking through the entire structure. The visual effect is of clouds of bacteria rising up through the biofilm. The water channels allow nutrients and waste to pass in and out of the biofilm, while the bacteria still remain protected within the slime coat.

Bacterial biofilms have become important clinically because of the marked resistance to antimicrobial agents that the biofilm bacteria display, relative to both their planktonic counterparts and from bacteria released from the confines of the biofilm. **Antibiotics** that swiftly kill the naked bacteria do not arm the biofilm bacteria, and may even promote the development of **antibiotic resistance**. Contributors to this resistance are likely the bacteria and the cocooning slime network.

Antibiotic resistant biofilms occur on artificial heart valves, urinary catheters, gallstones, and in the lungs of those afflicted with cystic fibrosis, as only a few examples. In the example of cystic fibrosis, the biofilm also acts to shield the *Pseudomonas aeruginosa* bacteria from the antibacterial responses of the host's **immune system**. The immune response may remain in place for a long time, which irritates and damages the lung tissue. This damage and the resulting loss of function can be lethal.

See also Anti-adhesion methods; Antibiotic resistance, tests for; Bacterial adaptation

BIOGEOCHEMICAL CYCLES

The term biogeochemical cycle refers to any set of changes that occur as a particular element passes back and forth between the living and non-living worlds. For example, carbon occurs sometimes in the form of an atmospheric gas (carbon dioxide), sometimes in rocks and minerals (limestone and marble), and sometimes as the key element of which all living organisms are made. Over time, chemical changes occur that convert one form of carbon to another form. At various points in the carbon cycle, the element occurs in living organisms and at other points it occurs in the Earth's atmosphere, lithosphere, or hydrosphere.

The universe contains about ninety different naturally occurring elements. Six elements, carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus, make up over 95% of the mass of all living organisms on Earth. Because the total amount of each element is essentially constant, some cycling process must take place. When an organism dies, for example, the elements of which it is composed continue to move through a cycle, returning to the Earth, to the air, to the ocean, or to another organism.

All biogeochemical cycles are complex. A variety of pathways are available by which an element can move among hydrosphere, lithosphere, atmosphere, and biosphere. For instance, nitrogen can move from the lithosphere to the atmosphere by the direct decomposition of dead organisms or by the reduction of nitrates and nitrites in the soil. Most changes in the nitrogen cycle occur as the result of bacterial action on one compound or another. Other cycles do not require the intervention of **bacteria**. In the sulfur cycle, for example, sulfur dioxide in the atmosphere can react directly with compounds in the earth to make new sulfur compounds that become part of the lithosphere. Those compounds can then be transferred directly to the biosphere by plants growing in the earth.

Most cycles involve the transport of an element through all four parts of the planet—hydrosphere, atmosphere, lithosphere, and biosphere. The phosphorous cycle is an exception since phosphorus is essentially absent from the atmosphere. It does move from biosphere to the lithosphere (when organisms die and decay) to the hydrosphere (when phosphorous-containing compounds dissolve in water) and back to the biosphere (when plants incorporate phosphorus from water).

Hydrogen and oxygen tend to move together through the planet in the hydrologic cycle. Precipitation carries water from the atmosphere to the hydrosphere and lithosphere. It then becomes part of living organisms (the biosphere) before being returned to the atmosphere through **respiration**, transpiration, and evaporation.

All biogeochemical cycles are affected by human activities. As fossil fuels are burned, for example, the transfer of carbon from a very old reserve (decayed plants and animals buried in the earth) to a new one (the atmosphere, as carbon dioxide) is accelerated. The long-term impact of this form of human activity on the global environment, as well as that of other forms, is not yet known. Some scientists assert, however, that those affects can be profound, resulting in significant climate changes far into the future.

See also Biodegradable substances; Carbon cycle in microorganisms; Composting, microbiological aspects; Economic uses and benefits of microorganisms; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Nitrogen cycle in microorganisms; Oxygen cycle in microorganisms

Under the proper conditions, physical phenomena such as lightning are capable of providing the energy needed for atoms and molecules to assemble into the fundamental building blocks of life.

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

Bioinformatics, or computational biology, refers to the development of new database methods to store genomic information, computational software programs, and methods to extract, process, and evaluate this information; it also refers to the refinement of existing techniques to acquire the genomic data. Finding genes and determining their function, predicting the structure of proteins and **RNA** sequences from the available **DNA** sequence, and determining the evolutionary relationship of proteins and DNA sequences are also part of bioinformatics.

The genome sequences of some **bacteria**, **yeast**, a nematode, the fruit fly *Drosophila* and several plants have been obtained during the past decade, with many more sequences nearing completion. During the year 2000, the sequencing of the human genome was completed. In addition to this accumulation of nucleotide sequence data, elucidation of the three-dimensional structure of proteins coded for by the genes has been accelerating. The result is a vast ever-increasing amount of databases and genetic information The efficient and productive use of this information requires the specialized computational techniques and software. Bioinformatics has developed and grown from the need to extract and analyze the reams of information pertaining to genomic information like nucleotide sequences and protein structure.

Bioinformatics utilizes statistical analysis, stepwise computational analysis and database management tools in order to search databases of DNA or protein sequences to filter out background from useful data and enable comparison of data from diverse databases. This sort of analysis is on-going. The exploding number of databases, and the various experimental methods used to acquire the data, can make comparisons tedious to achieve. However, the benefits can be enormous. The immense size and network of biological databases provides a resource to answer biological questions about mapping, **gene** expression patterns, molecular modeling, molecular **evolution**, and to assist in the structural-based design of therapeutic drugs.

Obtaining information is a multi-step process. Databases are examined, or browsed, by posing complex computational questions. Researchers who have derived a DNA or protein sequence can submit the sequence to public repositories of such information to see if there is a match or similarity with their sequence. If so, further analysis may reveal a putative structure for the protein coded for by the sequence as well as a putative function for that protein. Four primary databases, those containing one type of information (only DNA sequence

data or only protein sequence data), currently available for these purposes are the European **Molecular Biology** DNA Sequence Database (EMBL), GenBank, SwissProt and the Protein Identification Resource (PIR). Secondary databases contain information derived from other databases. Specialist databases, or knowledge databases, are collections of sequence information, expert commentary and reference literature. Finally, integrated databases are collections (amalgamations) of primary and secondary databases.

The area of bioinformatics concerned with the derivation of protein sequences makes it conceivable to predict three-dimensional structures of the protein molecules, by use of computer graphics and by comparison with similar proteins, which have been obtained as a crystal. Knowledge of structure allows the site(s) critical for the function of the protein to be determined. Subsequently, drugs active against the site can be designed, or the protein can be utilized to enhanced commercial production processes, such as in pharmaceutical bioinformatics.

Bioinformatics also encompasses the field of comparative genomics. This is the comparison of functionally equivalent genes across species. A yeast gene is likely to have the same function as a worm protein with the same amino acid. Alternately, genes having similar sequence may have divergent functions. Such similarities and differences will be revealed by the sequence information. Practically, such knowledge aids in the **selection** and design of genes to instill a specific function in a product to enhance its commercial appeal.

The most widely known example of a bioinformatics driven endeavor is the Human Genome Project. It was initiated in 1990 under the direction of the National Center for Human Genome Research with the goal of sequencing the entire human genome. While this has now been accomplished, the larger aim of determining the function of each of the approximately 50,000 genes in the human genome will require much further time and effort. Work related to the Human Genome Project has allowed dramatic improvements in molecular biological techniques and improved computational tools for studying genomic function.

See also Hazard Analysis and Critical Point Program (HAACP); Immunological analysis techniques; The Institute for Genomic Research (TIGR); Medical training and careers in microbiology; Transplantation genetics and immunology

BIOLOGICAL WARFARE

Biological warfare, as defined by The United Nations, is the use of any living organism (e.g. bacterium, virus) or an infective component (e.g., toxin), to cause disease or death in humans, animals, or plants. In contrast to **bioterrorism**, biological warfare is defined as the "state-sanctioned" use of biological weapons on an opposing military force or civilian population.

Biological weapons include **viruses**, **bacteria**, **rickettsia**, and biological toxins. Of particular concern are genetically altered **microorganisms**, whose effect can be made to be group-specific. In other words, persons with particular traits are susceptible to these microorganisms.

The use of biological weapons by armies has been a reality for centuries. For example, in ancient records of battles exist the documented use of diseased bodies and cattle that had died of microbial diseases to poison wells. There are even records that infected bodies or carcasses were catapulted into cities under siege.

In the earliest years of the twentieth century, however, weapons of biological warfare were specifically developed by modern methods, refined, and stockpiled by various governments.

During World War I, Germany developed a biological warfare program based on the **anthrax** bacillus (*Bacillus anthracis*) and a strain of *Pseudomonas* known as *Burkholderia mallei*. The latter is also the cause of Glanders disease in cattle.

Allied efforts in Canada, the United States, and Britain to develop anthrax-based weapons were also active in World War II During World War II, Britain actually produced five million anthrax cakes at the U.K. Chemical and Biological Defense Establishment at Porton Down facility that were intended to be dropped on Germany to infect the food chain. The weapons were never used. Against their will, prisoners in German Nazi concentration camps were maliciously infected with pathogens, such as **hepatitis** A, *Plasmodia* spp., and two types of *Rickettsia* bacteria, during studies allegedly designed to develop vaccines and antibacterial drugs. Japan also conducted extensive biological weapon research during World War II in occupied Manchuria, China. Unwilling prisoners were infected with a variety of pathogens, including *Neisseria meningitis*, *Bacillus anthracis*, *Shigella spp*, and *Yersinia pestis*. It has been estimated that over 10,000 prisoners died as a result of either infection or execution following infection. In addition, biological agents contaminated the water supply and some food items, and an estimated 15 million potentially plague-infected fleas were released from aircraft, affecting many Chinese cities. However, as the Japanese military found out, biological weapons have fundamental disadvantages: they are unpredictable and difficult to control. After infectious agents were let loose in China by the Japanese, approximately 10,000 illnesses and 1,700 deaths were estimated to have occurred among Japanese troops.

A particularly relevant example of a microorganism used in biological warfare is *Bacillus anthracis*. This bacterium causes anthrax. *Bacillus anthracis* can live as a vegetative cell, growing and dividing as bacteria normally do. The organism has also evolved the ability to withstand potentially lethal environmental conditions by forming a near-dormant, highly resistant form known as a spore. The spore is designed to hibernate until conditions are conducive for growth and reproduction. Then, the spore resuscitates and active metabolic life resumes. The spore form can be easily inhaled to produce a highly lethal inhalation anthrax. The spores quickly and easily resuscitate in the warm and humid conditions of the lung. Contact with spores can also produce a less lethal but dangerous cutaneous anthrax infection.

One of the "attractive" aspects of anthrax as a weapon of biological warfare is its ability to be dispersed over the enemy by air. Other biological weapons also have this capacity. The dangers of an airborne release of bioweapons are well documented. British open-air testing of anthrax weapons in 1941 on Gruinard Island in Scotland rendered the island inhabitable for five decades. The US Army conducted a study in 1951-52 called "Operation Sea Spray" to study wind currents that might carry biological weapons. As part of the project design, balloons were filled with *Serratia marcescens* (then thought to be harmless) and exploded over San Francisco. Shortly thereafter, there was a corresponding dramatic increase in reported **pneumonia** and urinary tract infections. And, in 1979, an accidental release of anthrax spores, a gram at most and only for several minutes, occurred at a bioweapons facility near the Russian city of Sverdlovsk. At least 77 people were sickened and 66 died. All the affected were some 4 kilometers downwind of the facility. Sheep and cattle up to 50 kilometers downwind became ill.

The first diplomatic effort to limit biological warfare was the Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare. This treaty, ratified in 1925, prohibited the use of biological weapons. The treaty has not been effective. For example, during the "Cold War" between the United States and the then Soviet Union in the 1950s and 1960s, the United States constructed research facilities to develop antisera, vaccines, and equipment for protection against a possible biological attack. As well, the use of microorganisms as offensive weapons was actively investigated.

Since then, other initiatives to ban the use of biological warfare and to destroy the stockpiles of biological weapons have been attempted. For example, in 1972 more than 100 countries, including the United States, signed the Convention on the Prohibition of the Development Production, and the Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Although the United States formally stopped biological weapons research in 1969 (by executive order of then President Richard M. Nixon), the Soviet Union carried on biological weapons research until its demise. Despite the international prohibitions, the existence of biological weapons remains dangerous reality.

See also Anthrax, terrorist use of as a biological weapon; Bacteria and bacterial infection; Bioterrorism, protective measures; Bioterrorism; Infection and resistance; Viruses and response to viral infection

Biological Weapons Convention (BWC)

The Biological Weapons Convention (more properly but less widely known as The Biological and Toxin Weapons Convention) is an international agreement that prohibits the development and stockpiling of biological weapons. The language of the Biological Weapons Convention (BWC) describes biological weapons as "repugnant to the conscience of mankind." Formulated in 1972, the treaty has been signed (as of June 2002) by more than 159 countries; 141 countries have formally ratified the BWC.

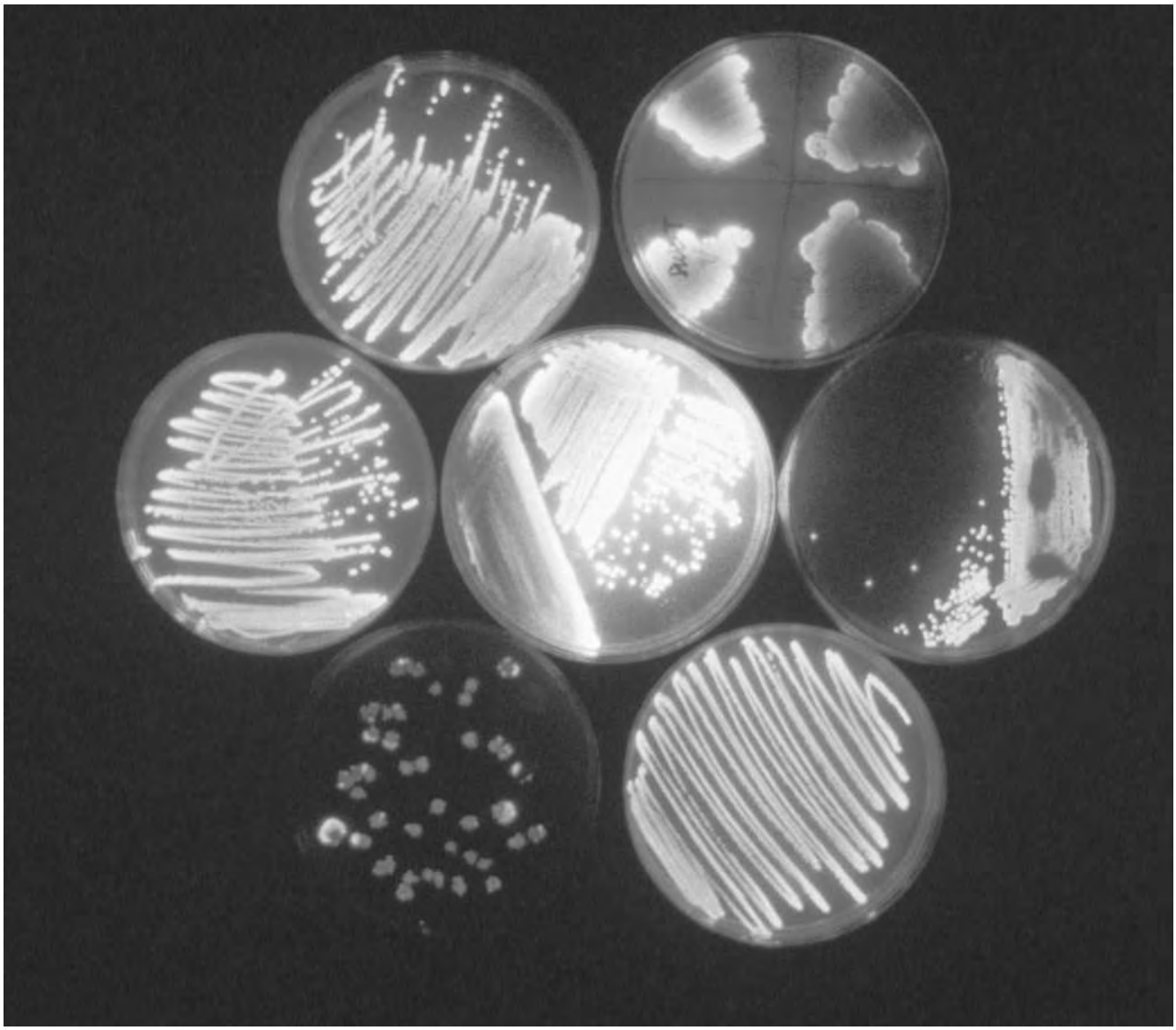
The BWC broadly prohibits the development of pathogens—disease-causing **microorganisms** such as **viruses** and bacteria—and biological toxins that do not have established prophylactic merit (i.e., no ability to serve a protective immunological role), beneficial industrial use, or use in medical treatment.

The United States renounced the first-use of biological weapons and restricted future weapons research programs to issues concerning defensive responses (e.g., **immunization**, detection, etc.), by executive order in 1969.

Although the BWC disarmament provisions stipulated that biological weapons stockpiles were to have been destroyed by 1975, most Western intelligence agencies openly question whether all stockpiles have been destroyed. Despite the fact that it was a signatory party to the 1972 Biological and Toxin Weapons Convention, the former Soviet Union maintained a well-funded and high-intensity biological weapons program throughout the 1970s and 1980s, producing and stockpiling biological weapons including **anthrax** and **smallpox** agents. US intelligence agencies openly raise doubt as to whether successor Russian biological weapons programs have been completely dismantled. In June 2002, traces of biological and chemical weapon agents were found in Uzbekistan on a military base used by U.S. troops fighting in Afghanistan. Early analysis dates and attributes the source of the **contamination** to former Soviet Union or successor Russian biological and chemical weapons programs that utilized the base.

As of 2002, intelligence estimates compiled from various agencies provide indications that more than two dozen countries are actively involved in the development of biological weapons. The US Office of Technology Assessment and the United States Department of State have identified a list of potential enemy states developing biological weapons. Such potentially hostile nations include Iran, Iraq, Libya, Syria, North Korea, and China.

The BWC prohibits the offensive weaponization of biological agents (e.g., anthrax spores). The BWC also prohibits the **transformation** of biological agents with established legitimate and sanctioned purposes into agents of a nature and quality that could be used to effectively induce illness or death. In addition to offensive weaponization of microorganisms or toxins, prohibited research procedures include concentrating a strain of bacterium or virus, altering the size of aggregations of potentially harmful biologic agents (e.g., refining anthrax spore sizes to spore sizes small enough to be effectively and widely carried in air currents), producing strains capable of withstanding normally adverse environmental conditions (e.g., disbursement weapons blast), and the manipulation of a number of other factors that make biologic agents effective weapons.



Bioluminescent bacteria.

Although there have been several international meetings designed to strengthen the implementation and monitoring of BWC provisions, BWC verification procedures are currently the responsibility of an ad hoc commission of scientists. Broad international efforts to coordinate and strengthen enforcement of BWC provisions remains elusive.

See also Anthrax, terrorist use of as a biological weapon; Bacteria and bacterial infection; Biological warfare; Epidemics and pandemics; Vaccine

BIOLOGY, CENTRAL DOGMA OF • *see* MOLECULAR BIOLOGY AND MOLECULAR GENETICS

BIOLUMINESCENCE

Bioluminescence is the production of light by living organisms. Some single-celled organisms (**bacteria** and protista) as well as many multicellular animals and **fungi** demonstrate bioluminescence.

Light is produced by most bioluminescent organisms when a chemical called luciferin reacts with oxygen to produce light and oxyluciferin. The reaction between luciferin and oxygen is catalyzed by the enzyme luciferase. Luciferases, like luciferins, usually have different chemical structures in different organisms. In addition to luciferin, oxygen, and luciferase, other molecules (called cofactors) must be present for the bioluminescent reaction to proceed. Cofactors are molecules required by an enzyme (in this case luciferase)

to perform its catalytic function. Common cofactors required for bioluminescent reactions are calcium and ATP, a molecule used to store and release energy that is found in all organisms.

The terms luciferin and luciferase were first introduced in 1885. The German scientist Emil du Bois-Reymond obtained two different extracts from bioluminescent clams and beetles. When Dubois mixed these extracts they produced light. He also found that if one of these extracts was first heated, no light would be produced upon mixing. Heating the other extract had no effect on the reaction, so Dubois concluded that there were at least two components to the reaction. Dubois hypothesized that the heat-resistant chemical undergoes a chemical change during the reaction, and called this compound luciferin. The heat sensitive chemical, Dubois concluded, was an enzyme which he called luciferase.

The two basic components needed to produce a bioluminescent reaction, luciferin and luciferase, can be isolated from the organisms that produce them. When they are mixed in the presence of oxygen and the appropriate cofactors, these components will produce light with an intensity dependent on the quantity of luciferin and luciferase added, as well as the oxygen and cofactor concentrations. Luciferases isolated from fireflies and other beetles are commonly used in research.

Scientists have used isolated luciferin and luciferase to determine the concentrations of important biological molecules such as ATP and calcium. After adding a known amount of luciferin and luciferase to a blood or tissue sample, the cofactor concentrations may be determined from the intensity of the light emitted. Scientists have also found numerous other uses for the bioluminescent reaction such as using it to quantify specific molecules that do not directly participate in the bioluminescence reaction. To do this, scientists attach luciferase to antibodies—molecules produced by the **immune system** that bind to specific molecules called antigens. The antibody-luciferase complex is added to a sample where it binds to the molecule to be quantified. Following washing to remove unbound antibodies, the molecule of interest can be quantified indirectly by adding luciferin and measuring the light emitted. Methods used to quantify particular compounds in biological samples such as the ones described here are called assays.

In recent studies, luciferase has been used to study viral and bacterial infections in living animals and to detect bacterial contaminants in food. The luciferase reaction also is used to determine **DNA** sequences, the order of the four types of molecules that comprise DNA and code for proteins.

Luciferase is often used as a "reporter gene" to study how individual genes are activated to produce protein or repressed to stop producing protein. Most genes are turned on and off by DNA located in front of the part of the **gene** that codes for protein. This region is called the gene promoter. A specific gene promoter can be attached to the DNA that codes for firefly luciferase and introduced into an organism. The activity of the gene promoter can then be studied by measuring the bioluminescence produced in the luciferase reaction. Thus, the luciferase gene can be used to "report" the activity of a promoter for another gene.

Bioluminescent organisms in the terrestrial environment include species of fungi and insects. The most familiar of these is the firefly, which can often be seen glowing during the warm summer months. In some instances organisms use bioluminescence to communicate, such as in fireflies, which use light to attract members of the opposite sex. Marine environments support a number of bioluminescent organisms including species of bacteria, **dinoflagellates**, jellyfish, coral, shrimp, and fish. On any given night one can see the luminescent sparkle produced by the single-celled dinoflagellates when water is disturbed by a ship's bow or a swimmer's motions.

See also Antibiotic resistance, tests for; Biotechnology; Food safety; Immunoflorescence; Microbial genetics

BIOREMEDIATION

Bioremediation is the use of living organisms or ecological processes to deal with a given environmental problem. The most common use of bioremediation is the metabolic breakdown or removal of toxic chemicals before or after they have been discharged into the environment. This process takes advantage of the fact that certain **microorganisms** can utilize toxic chemicals as metabolic substrates and render them into less toxic compounds. Bioremediation is a relatively new and actively developing technology. Increasingly, microorganisms and plants are being genetically engineered to aide in their ability to remove deleterious substances.

In general, bioremediation methodologies focus on one of two approaches. The first approach, bioaugmentation, aims to increase the abundance of certain species or groups of microorganisms that can metabolize toxic chemicals. Bioaugmentation involves the deliberate addition of strains or species of microorganisms that are effective at treating particular toxic chemicals, but are not indigenous to or abundant in the treatment area. Alternatively, environmental conditions may be altered in order to enhance the actions of such organisms that are already present in the environment. This process is known as biostimulation and usually involves fertilization, aeration, or irrigation. Biostimulation focuses on rapidly increasing the abundance of naturally occurring microorganisms capable of dealing with certain types of environmental problems.

Accidental spills of petroleum or other hydrocarbons on land and water are regrettable but frequent occurrences. Once spilled, petroleum and its various refined products can be persistent environmental contaminants. However, these organic chemicals can also be metabolized by certain microorganisms, whose processes transform the toxins into more simple compounds, such as carbon dioxide, water, and other inorganic chemicals. In the past, concentrates of **bacteria** that are highly efficient at metabolizing hydrocarbons have been "seeded" into spill areas in an attempt to increase the rate of degradation of the spill residues. Although this technique has occasionally been effective, it commonly fails because the large concentrations of hydrocarbons stimulates rapid growth of indigenous microorganisms also capable of utilizing hydrocarbons as metabolic substrates. Consequently, seeding of microorgan-

An oil spill. The oil does not mix with the water.

isms that are metabolically specific to hydrocarbons often does not affect the overall rate of degradation.

Environmental conditions under which spill residues occur are often sub-optimal for toxin degradation by microorganisms. Most commonly the rate is limited by the availability of oxygen or of certain nutrients such as nitrate and phosphate. Therefore the microbial breakdown of spilled hydrocarbons on land can be greatly enhanced by aeration and fertilization of the soil.

Metals are common pollutants of water and land because they are emitted by many industrial, agricultural, and domestic sources. In some situations organisms can be utilized to concentrate metals that are dispersed in the environment. For example, metal-polluted waste waters can be treated by encouraging the vigorous growth of certain types of vascular plants. This bioremediation system, also known as phytoremediation, works because the growing plants accumulate high levels of metals in their shoots, thereby reducing the concentration in the water to a more tolerable range. The plants can then be harvested to remove the metals from the system.

Many advanced sewage-treatment technologies utilize microbial processes to oxidize organic matter associated with fecal wastes and to decrease concentrations of soluble compounds or ions of metals, pesticides, and other toxic chemicals. Decreasing the aqueous concentrations of toxic chemicals is accomplished by a combination of chemical adsorption as well as microbial biodegradation of complex chemicals into their inorganic constituents.

If successful, bioremediation of contaminated sites can offer a cheaper, less environmentally damaging alternative to traditional clean-up technologies.

See also Economic uses and benefits of microorganisms; Microbial genetics; Waste water treatment; Water purification; Water quality

BIOTECHNOLOGY

The word biotechnology was coined in 1919 by Karl Ereky to apply to the interaction of biology with human technology. Today, it comes to mean a broad range of technologies from genetic engineering (recombinant **DNA** techniques), to animal breeding and industrial **fermentation**. Accurately, biotechnology is defined as the integrated use of **biochemistry**, microbiology, and engineering sciences in order to achieve technological (industrial) application of the capabilities of **microorganisms**, cultured tissue cells, and parts thereof.

The nature of biotechnology has undergone a dramatic change in the last half century. Modern biotechnology is

greatly based on recent developments in **molecular biology**, especially those in genetic engineering. Organisms from **bacteria** to cows are being genetically modified to produce pharmaceuticals and foods. Also, new methods of disease **gene** isolation, analysis, and detection, as well as gene therapy, promise to revolutionize medicine.

In theory, the steps involved in genetic engineering are relatively simple. First, scientists decide the changes to be made in a specific DNA molecule. It is desirable in some cases to alter a human DNA molecule to correct errors that result in a disease such as diabetes. In other cases, researchers might add instructions to a DNA molecule that it does not normally carry: instructions for the manufacture of a chemical such as insulin, for example, in the DNA of bacteria that normally lack the ability to make insulin. Scientists also modify existing DNA to correct errors or add new information. Such methods are now well developed. Finally, scientists look for a way to put the recombinant DNA molecule into the organisms in which it is to function. Once inside the organism, the new DNA molecule give correct instructions to cells in humans to correct genetic disorders, in bacteria (resulting in the production of new chemicals), or in other types of cells for other purposes.

Genetic engineering has resulted in a number of impressive accomplishments. Dozens of products that were once available only from natural sources and in limited amounts are now manufactured in abundance by genetically engineered microorganisms at relatively low cost. Insulin, human growth hormone, tissue plasminogen activator, and alpha interferon are examples. In addition, the first trials with the alteration of human DNA to cure a genetic disorder began in 1991.

Molecular geneticists use molecular **cloning** techniques on a daily basis to replicate various genetic materials such as gene segments and cells. The process of molecular cloning involves isolating a DNA sequence of interest and obtaining multiple copies of it in an organism that is capable of growth over extended periods. Large quantities of the DNA molecule can then be isolated in pure form for detailed molecular analysis. The ability to generate virtually endless copies (clones) of a particular sequence is the basis of recombinant DNA technology and its application to human and medical genetics.

A technique called positional cloning is used to map the location of a human disease gene. Positional cloning is a relatively new approach to finding genes. A particular DNA marker is linked to the disease if, in general, family members with certain nucleotides at the marker always have the disease, and family members with other nucleotides at the marker do not have the disease. Once a suspected linkage result is confirmed, researchers can then test other markers known to map close to the one found, in an attempt to move closer and closer to the disease gene of interest. The gene can then be cloned if the DNA sequence has the characteristics of a gene and it can be shown that particular **mutations** in the gene confer disease.

Embryo cloning is another example of genetic engineering. Agricultural scientists are experimenting with embryo cloning processes with animal embryos to improve upon and increase the production of livestock. The first successful attempt at producing live animals by embryo cloning was reported by a research group in Scotland on March 6, 1997.

Although genetic engineering is a very important component of biotechnology, it is not alone. Biotechnology has been used by humans for thousands of years. Some of the oldest manufacturing processes known to humankind make use of biotechnology. Beer, wine, and bread making, for example, all occur because of the process of fermentation. As early as the seventeenth century, bacteria were used to remove copper from its ores. Around 1910, scientists found that bacteria could be used to decompose organic matter in sewage. A method that uses microorganisms to produce glycerol synthetically proved very important in the World War I since glycerol is essential to the manufacture of explosives.

See also Fermentation; Immune complex test; Immunoelectrophoresis; Immunofluorescence; Immunogenetics; Immunologic therapies; Immunological analysis techniques; Immunosuppressant drugs; *In vitro* and *in vivo* research

BIOTERRORISM

Bioterrorism is the use of a biological weapon against a civilian population. As with any form of terrorism, its purposes include the undermining of morale, creating chaos, or achieving political goals. Biological weapons use **microorganisms** and toxins to produce disease and death in humans, livestock, and crops.

Biological, chemical, and nuclear weapons can all be used to achieve similar destructive goals, but unlike chemical and nuclear technologies that are expensive to create, biological weapons are relatively inexpensive. They are easy to transport and resist detection by standard security systems. In general, chemical weapons act acutely, causing illness in minutes to hours at the scene of release. For example, the release of sarin gas by the religious sect Aum Shinrikyo in the Tokyo subway in 1995 killed 12 and hospitalized 5,000 people. In contrast, the damage from biological weapons may not become evident until weeks after an attack. If the pathogenic (disease-causing) agent is transmissible, a bioterrorist attack could eventually kill thousands over a much larger area than the initial area of attack.

Bioterrorism can also be enigmatic, destructive, and costly even when targeted at a relatively few number of individuals. Starting in September 2001, bioterrorist attacks with anthrax-causing **bacteria** distributed through the mail targeted only a few U.S. government leaders, media representatives, and seemingly random private citizens. As of June 2002, these attacks remain unsolved. Regardless, in addition to the tragic deaths of five people, the terrorist attacks cost the United States millions of dollars and caused widespread concern. These attacks also exemplified the fact that bioterrorism can strike at the political and economic infrastructure of a targeted country.

Although the deliberate production and stockpiling of biological weapons is prohibited by the 1972 **Biological Weapons Convention** (BWC)—the United States stopped for-

A decontamination crew responds to a possible release of anthrax by terrorists at a United States postal facility in 2001.

mal bioweapons programs in 1969—unintended byproducts or deliberate misuse of emerging technologies offer potential bioterrorists opportunities to prepare or refine biogenic weapons. Genetic engineering technologies can be used to produce a wide variety of bioweapons, including organisms that produce toxins or that are more weaponizable because they are easier to aerosolize (suspend as droplets in the air). More conventional laboratory technologies can also produce organisms resistant to **antibiotics**, routine vaccines, and therapeutics. Both technologies can produce organisms that cannot be detected by antibody-based sensor systems.

Among the most serious of potential bioterrorist weapons are those that use **smallpox** (caused by the **Variola virus**), **anthrax** (caused by *Bacillus anthracis*), and plague (caused by *Yersinia pestis*). During naturally occurring **epidemics** throughout the ages, these organisms have killed significant portions of afflicted populations. With the advent of vaccines and antibiotics, few U.S. physicians now have the experience to readily recognize these diseases, any of which could cause catastrophic numbers of deaths.

Although the last case of smallpox was reported in Somalia in 1977, experts suspect that smallpox **viruses** may be in the biowarfare laboratories of many nations around the world. At present, only two facilities—one in the United States and one in Russia—are authorized to store the virus. As recently as 1992, United States intelligence agencies learned that Russia had the ability to launch missiles containing weapons-grade smallpox at major cities in the U.S. A number of terrorist organizations—including the radical Islamist Al Qaeda terrorist organization—actively seek the acquisition of state-sponsored research into weapons technology and pathogens.

There are many reasons behind the spread of biowarfare technology. Prominent among them are economic incentives; some governments may resort to selling bits of scientific information that can be pieced together by the buyer to create biological weapons. In addition, scientists in politically repressive or unstable countries may be forced to participate in research that eventually ends up in the hands of terrorists.

A biological weapon may ultimately prove more powerful than a conventional weapon because its effects can be far-reaching and uncontrollable. In 1979, after an accident involving *B. anthracis* in the Soviet Union, doctors reported civilians dying of anthrax **pneumonia** (i.e., inhalation anthrax). Death from anthrax pneumonia is usually swift. The bacilli multiply rapidly and produce a toxin that causes breathing to stop. While antibiotics can combat this bacillus, supplies adequate to meet the treatment needs following an attack on a large urban population would need to be delivered and

distributed within 24 to 48 hours of exposure. The National Pharmaceutical Stockpile Program (NPS) is designed to enable such a response to a bioterrorist attack.

Preparing a strategy to defend against these types of organisms, whether in a natural or genetically modified state, is difficult. Some of the strategies include the use of bacterial **RNA** based on structural templates to identify pathogens; increased abilities for rapid **genetic identification of microorganisms**; developing a database of virtual pathogenic molecules; and development of antibacterial molecules that attach to pathogens but do not harm humans or animals. Each of these is an attempt to increase—and make more flexible—identification capabilities.

Researchers are also working to counter potential attacks using several innovative technological strategies. For example, promising research is being done with biorobots or microchip-mechanized insects, which have computerized artificial systems that mimic biological processes such as neural networks, can test responses to substances of biological or chemical origin. These insects can, in a single operation, process **DNA**, screen blood samples, scan for disease genes, and monitor genetic cell activity. The robotics program of the Defense Advanced Research Project (DARPA) works to rapidly identify bio-responses to pathogens, and to design effective and rapid treatment methods.

Biosensor technology is the driving force in the development of biochips for detection of biological and chemical contaminants. Bees, beetles, and other insects outfitted with sensors are used to collect real-time information about the presence of toxins or similar threats. Using fiber optics or electrochemical devices, biosensors have detected microorganisms in chemicals and foods, and they offer the promise of rapid identification of biogenic agents following a bioterrorist attack. The early accurate identification of biogenic agents is critical to implementing effective response and treatment protocols.

To combat biological agents, bioindustries are developing a wide range of antibiotics and vaccines. In addition, advances in **bioinformatics** (i.e., the computerization of information acquired during, for example, genetic screening) also increases flexibility in the development of effective counters to biogenic weapons.

In addition to detecting and neutralizing attempts to weaponize biogenic agents (i.e., attempts to develop bombs or other instruments that could effectively disburse a bacterium or virus), the major problem in developing effective counter strategies to bioterrorist attacks involves the breadth of organisms used in **biological warfare**. For example, researchers are analyzing many pathogens in an effort to identify common genetic and cellular components. One strategy is to look for common areas or vulnerabilities in specific sites of DNA, RNA, or proteins. Regardless of whether the pathogens evolve naturally or are engineered, the identification of common traits will assist in developing counter measures (i.e., specific vaccines or antibiotics).

See also Anthrax, terrorist use of as a biological weapon; Biological warfare; Contamination, bacterial and viral; Genetic identification of microorganisms; Public health, current issues

BIOTERRORISM, IDENTIFICATION OF MICROORGANISMS

• *see* GENETIC IDENTIFICATION OF MICROORGANISMS

BIOTERRORISM, PROTECTIVE MEASURES

In the aftermath of the September 11, 2001 terrorist attacks on the United States and the subsequent anthrax attacks on U.S. government officials, media representatives, and citizens, the development of measures to protect against biological terrorism became an urgent and contentious issue of public debate. Although the desire to increase readiness and response capabilities to possible nuclear, chemical, and biological attacks is widespread, consensus on which preventative measures to undertake remains elusive.

The evolution of political realities in the last half of the twentieth century and events of 2001 suggest that, within the first half of the twenty-first century, biological weapons will surpass nuclear and chemical weapons as a threat to the citizens of the United States.

Although a range of protective options exists—from the stockpiling of **antibiotics** to the full-scale resumption of biological weapons programs—no single solution provides comprehensive protection to the complex array of potential biological agents that might be used as terrorist weapons. Many scientists argue, therefore, that focusing on one specific set of protective measures (e.g., broadly inoculating the public against the virus causing **smallpox**) might actually lower overall preparedness and that a key protective measure entails upgrading fundamental research capabilities.

The array of protective measures against **bioterrorism** are divided into strategic, tactical, and personal measures.

Late in 2001, the United States and its NATO (North Atlantic Treaty Organization) allies reaffirmed treaty commitments that stipulate the use of any weapon of mass destruction (i.e., biological, chemical, or nuclear weapons) against any member state would be interpreted as an attack against all treaty partners. As of June 2002, this increased strategic deterrence was directed at Iraq and other states that might seek to develop or use biological weapons—or to harbor or aid terrorists seeking to develop weapons of mass destruction. At the tactical level, the United States possesses a vast arsenal of weapons designed to detect and eliminate potential biological weapons. Among the tactical non-nuclear options is the use of precision-guided conventional thermal fuel-air bombs capable of destroying both biological research facilities and biologic agents.

Because terrorist operations are elusive, these large-scale military responses offer protection against only the largest, identifiable, and targetable enemies. They are largely ineffective against small, isolated, and dispersed "cells" of hostile forces, which operate domestically or within the borders of other nations. When laboratories capable of producing low-grade weaponizable anthrax-causing spores can be established in the basement of a typical house for less than $10,000,

Bioterrorist attack on the U.S. Capitol Building in 2001.

the limitations of full-scale military operations become apparent.

Many scientists and physicians argue that the most extreme of potential military responses, the formal resumption of biological weapons programs—even with a limited goal of enhancing understanding of potential biological agents and weapons delivery mechanisms—is unneeded and possibly detrimental to the development of effective protective measures. Not only would such a resumption be a violation of the **Biological Weapons Convention** to which the United States is a signatory and which prohibits such research, opponents of such a resumption argue any such renewal of research on biological weapons will divert critical resources, obscure needed research, and spark a new global biological arms race.

Most scientific bodies, including the National Institutes of Health, **Centers for Disease Control** and Prevention, advocate a balanced scientific and medical response to the need to develop protective measures against biological attack. Such plans allow for the maximum flexibility in terms of effective response to a number of disease causing pathogens.

In addition to increased research, preparedness programs are designed to allow a rapid response to the terrorist use of biological weapons. One such program, the National Pharmaceutical Stockpile Program (NPS) provides for a ready supply of antibiotics, vaccines, and other medical treatment countermeasures. The NPS stockpile is designed to be rapidly deployable to target areas. For example, in response to potential exposures to the *Bacillus anthracis* (the bacteria that causes anthrax) during the 2001 terrorist attacks, the United States government and some state agencies supplied Cipro, the antibiotic treatment of choice, to those potentially exposed to the bacterium. In addition to increasing funding for the NPS, additional funds have already been authorized to increase funding to train medical personnel in the early identification and treatment of disease caused by the most likely pathogens.

Despite this increased commitment to preparedness, medical exerts express near unanimity in doubting whether any series of programs or protocols can adequately provide comprehensive and effective protection to biological terrorism. Nonethless, advocates of increased research capabilities argue that laboratory and hospital facilities must be expanded and improved to provide maximum scientific flexibility in the identification and response to biogenic threats. For example, the Centers for Disease Control and Prevention (CDC), based in Atlanta, Georgia, has established a bioterrorism response program that includes increased testing and treatment capac-

ity. The CDC plan also calls for an increased emphasis on epidemiological detection and surveillance, along with the development of a public heath infrastructure capable of providing accurate information and treatment guidance to both medical professionals and the general public.

Because an informed and watchful public is key element in early detection of biological pathogens, the CDC openly identifies potential biological threats and publishes a list of those biological agents most likely to be used on its web pages. As of July 2002, the CDC identified approximately 36 microbes including **Ebola virus** variants and plague bacterium, that might be potentially used in a bioterrorist attack

Other protective and emergency response measures include the development of the CDC Rapid Response and Advanced Technology Laboratory, a Health Alert Network (HAN), National Electronic Data Surveillance System (NEDSS), and Epidemic Information Exchange (Epi-X) designed to coordinate information exchange in efforts to enhance early detection and identification of biological weapons.

Following the September 11, 2001 terrorist attacks on the United States, additional funds were quickly allocated to enhance the United States Department of Health and Human Services 1999 Bioterrorism Initiative. One of the key elements of the Bioterrorism Preparedness and Response Program (BPRP) increases the number and capacity of laboratory test facilities designed to identify pathogens and find effective countermeasures. In response to a call from the Bush administration, in December 2001, Congress more than doubled the previous funding for bioterrorism research.

Advances in effective therapeutic treatments are fundamentally dependent upon advances in the basic biology and pathological mechanisms of **microorganisms**. In response to terrorist attacks, in February 2002, the US National Institute of Allergy and Infectious Diseases (NIAID) established a group of experts to evaluate changes in research in order to effectively anticipate and counter potential terrorist threats. As a result, research into smallpox, anthrax, **botulism**, plague, **tularemia**, and viral **hemorrhagic fevers** is now given greater emphasis.

In addition to medical protective measures, a terrorist biological weapon attack could overburden medical infrastructure (e.g., cause an acute shortage of medical personnel and supplies) and cause economic havoc. It is also possible that an effective biological weapon could have no immediate effect upon humans, but could induce famine in livestock or ruin agricultural production. A number of former agreements between federal and state governments involving response planning will be subsumed by those of the Department of Homeland Security.

On a local level, cities and communities are encouraged to develop specific response procedures in the event of bioterrorism. Most hospitals are now required to have response plans in place as part of their accreditation requirements.

In addition to airborne and surface exposure, biologic agents may be disseminated in water supplies. Many communities have placed extra security on water supply and treatment facilities. The U.S. Environmental Protection Agency (EPA) has increased monitoring and working with local water suppliers to develop emergency response plans.

Although it is beyond the scope of this article to discuss specific personal protective measures—nor given the complexities and ever-changing threat would it be prudent to offer such specific medical advice—there are a number of general issues and measures that can be discussed. For example, the public has been specifically discouraged from buying often antiquated military surplus gas masks, because they can provide a false sense of protection. In addition to issues of potency decay, the hoarding of antibiotics has is also discouraged because inappropriate use can lead to the development of bacterial resistance and a consequential lowering of antibiotic effectiveness.

Generally, the public is urged to make provisions for a few days of food and water and to establish a safe room in homes and offices that can be temporarily sealed with duct tape to reduce outside air infiltration.

More specific response plans and protective measures are often based upon existing assessments of the danger posed by specific diseases and the organisms that produce the disease. For example, anthrax (*Bacillus anthracis*), botulism (*Clostridium botulinum* toxin), plague (*Yersinia pestis)*, smallpox (*Variola major)*, tularemia (*Francisella tularensis)*, and viral hemorrhagic fevers (e.g., Ebola, Marburg), and arenaviruses (e.g., Lassa) are considered high-risk and high-priority. Although these biogenic agents share the common attributes of being easily disseminated or transmitted and all can result in high mortality rates, the disease and their underlying microorganisms are fundamentally different and require different response procedures.

Two specific protective measures, smallpox and anthrax vaccines, remain highly controversial. CDC has adopted a position that, in the absence of a confirmed case of smallpox, the risks of resuming general smallpox **vaccination** far outweigh the potential benefits. In addition, **vaccine** is still maintained and could be used in the event of a bioterrorist emergency. CDC has also accelerated production of a smallpox vaccine. Moreover, vaccines delivered and injected during the incubation period for smallpox (approximately 12 days) convey at least some protection from the ravages of the disease.

Also controversial remains the safety and effectiveness of an anthrax vaccine used primarily by military personnel.

See also Anthrax, terrorist use of as a biological weapon; Bacteria and bacterial infection; Biological warfare; Epidemics and pandemics; Vaccine

BLACK DEATH • *see* BUBONIC PLAGUE

BLACK LIPID BILAYER MEMBRANE • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

BLACK SMOKER BACTERIA • *see* EXTREMOPHILES

BLOOD AGAR, HEMOLYSIS, AND HEMOLYTIC REACTIONS

Blood **agar** is a solid growth medium that contains red blood cells. The medium is used to detect **bacteria** that produce **enzymes** to break apart the blood cells. This process is also termed hemolysis. The degree to which the blood cells are hemolyzed is used to distinguish bacteria from one another.

The blood agar medium is prepared in a two-step process. First, a number of ingredients are added to water, including heart infusion, peptone, and sodium chloride. This solution is sterilized. Following **sterilization**, a known amount of sterile blood is added. The blood can be from rabbit or sheep. Rabbit blood is preferred if the target bacterium is from the group known as group A *Streptococcus*. Sheep blood is preferred if the target bacterium is *Haemophilus parahaemolyticus*.

Blood agar is a rich food source for bacteria. So, it can be used for primary culturing, that is, as a means of obtaining as wide a range of **bacterial growth** from a sample as possible. It is typically not used for this purpose, however, due to the expense of the medium. Other, less expensive agars will do the same thing. What blood agar is uniquely suited for is the determination of hemolysis.

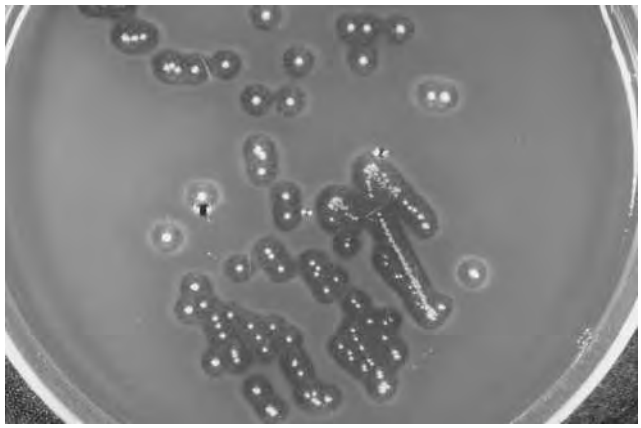
Hemolysis is the break down of the membrane of red blood cells by a bacterial protein known as hemolysin, which causes the release of hemoglobin from the red blood cell. Many types of bacterial posses hemolytic proteins. These proteins are thought to act by integrating into the membrane of the red blood cell and either punching a hole through the membrane or disrupting the structure of the membrane in some other way. The exact molecular details of hemolysin action is still unresolved.

The blood used in the agar is also treated beforehand to remove a molecule called fibrin, which participates in the clotting of blood. The absence of fibrin ensures that clotting of the blood does not occur in the agar, which could interfere with the visual detection of the hemolytic reactions.

There are three types of hemolysis, designated alpha, beta and gamma. Alpha hemolysis is a greenish discoloration that surrounds a bacterial **colony** growing on the agar. This type of hemolysis represents a partial decomposition of the hemoglobin of the red blood cells. Alpha hemolysis is characteristic of *Streptococcus pneumonia* and so can be used as a diagnostic feature in the identification of the bacterial strain.

Beta hemolysis represents a complete breakdown of the hemoglobin of the red blood cells in the vicinity of a bacterial colony. There is a clearing of the agar around a colony. Beta hemolysis is characteristic of *Streptococcus pyogenes* and some strains of*Staphylococcus aureus*.

The third type of hemolysis is actually no hemolysis at all. Gamma hemolysis is a lack of hemolysis in the area around a bacterial colony. A blood agar plate displaying gamma hemolysis actually appears brownish. This is a normal reaction of the blood to the growth conditions used (37° C in the presence of carbon dioxide). Gamma hemolysis is a characteristic of *Enterococcus faecalis*.



Beta hemolysis produced on blood agar by *Streptococcus viridans*.

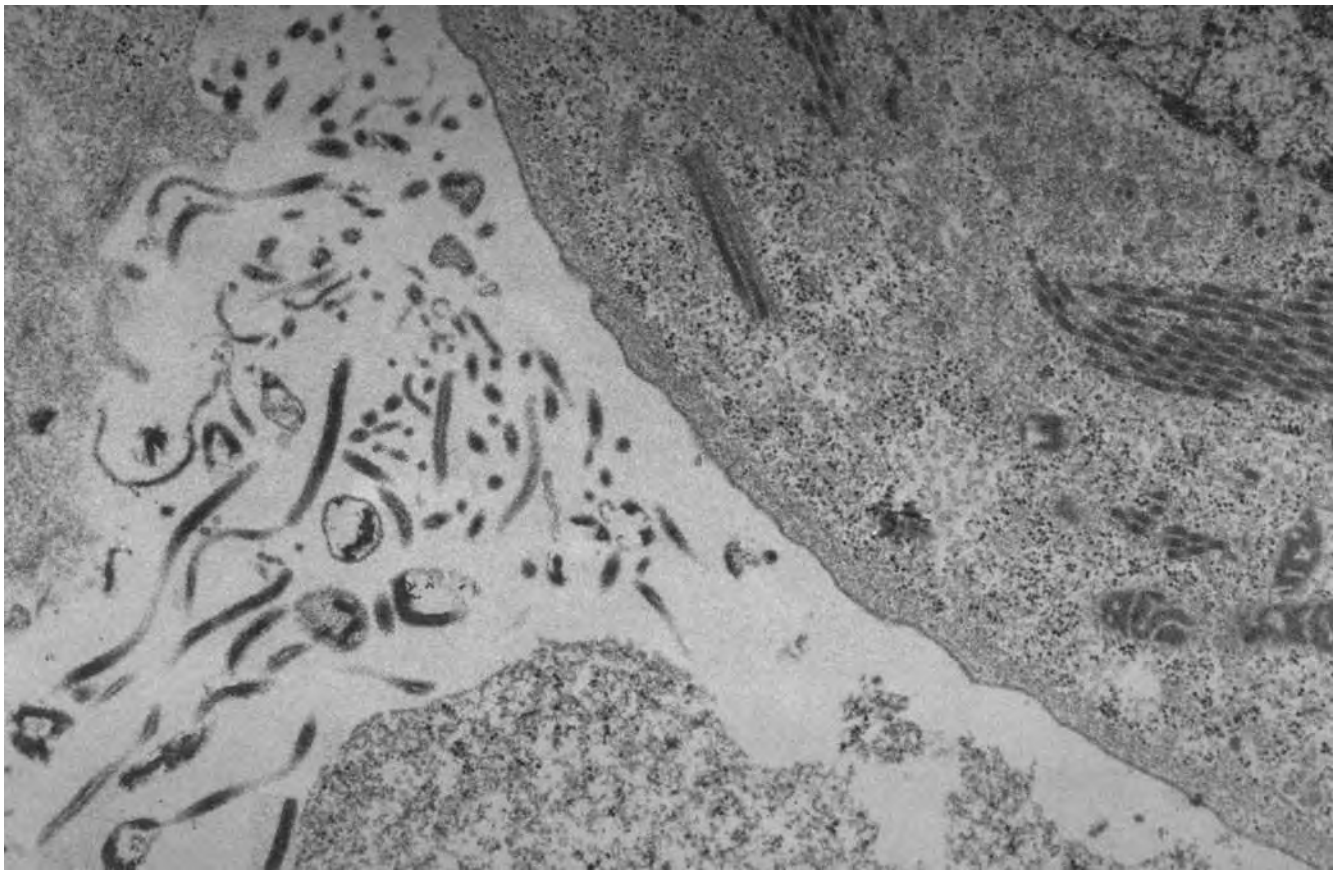
Hemolytic reactions can also display some synergy. That is, the combination of reactions produces a reaction that is stronger than either reaction alone. Certain species of bacteria, such as group B Strep (n example is *Streptococcus agalactiae*) are weakly beta-hemolytic. However, if the bacteria are in close proximity with a strain of Staphylococcus the beta-hemolysins of the two organisms can combine to produce an intense beta hemolytic reaction. This forms the basis of a test called the CAMP test (after the initials of its inventors).

The determination of hemolysis and of the hemolytic reactions is useful in distinguishing different types of bacteria. Subsequent biochemical testing can narrow down the identification even further. For example, a beta hemolytic reaction is indicative of a Streptococcus. Testing of the Streptococcus organisms with bacitracin is often the next step. Bacitracin is an antimicrobial that is produced by the bacterium *Bacillus subtilis*. *Streptococcus pyogenes* strains are almost uniformally sensitive to bacitracin. But other antigenic groups of Streptococcus are not bacitracin sensitive.

See also Laboratory techniques in microbiology; Staphylococci and staphylococcal infections; Streptococci and streptococcal infections

BLOOD BORNE INFECTIONS

Blood borne infections are those in which the infectious agent is transmitted from one person to another in contaminated blood. Infections of the blood can occur as a result of the spread of an ongoing infection, such as with **bacteria** including bacteria such as *Yersinia pestis*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. However, the latter re considered to be separate from true blood-borne infections.



Thin section electron micrograph of Ebola virus.

Bacterial blood borne infection can occur, typically in the transfusion of blood. Such infections arise from the **contamination** of the site of transfusion. While information on the rate of such infections is scarce, the risk of transmission of bacterial infections via transfusions is thought to be at least equal to the risk of viral infection. For example, figures from the United States Food and Drug Administration indicate that bacterial infections comprise at least 10% of transfusion-related deaths in the United States each year.

Another route of entry for bacteria are catheters. For example, it has been estimated that the chances of acquiring a urinary tract infection (which can subsequently spread to the blood) rises by up to 10% for each day a hospitalized patient is catheterized.

While bacteria can be problematic in blood borne infections, the typical agents of concern in blood borne infections are **protozoa** and **viruses**. The protozoan *Trypanosoma brucei* is transmitted to humans by the bite of the tsetse fly. The subsequent infection of the blood and organs of the body produces **sleeping sickness**, al illness that still afflicts millions each year in the underdeveloped world.

With respect to viral blood borne diseases, **hepatitis** A, hepatitis C, and the **Human Immunodeficiency Virus** (**HIV**; the cause of acquired **immunodeficiency** syndrome) are the focus of scrutiny in blood donors and in the setting of a hospital. Exposure to the blood from an infected person or the sharing of needles among intravenous drug users can transmit these viruses from person to person. In Canada, the contamination of donated blood and blood products with the hepatitis viruses and HIV in the 1980s sickened thousands of people. As a result the system for blood donation and the monitoring guidelines for the blood and blood products was completely overhauled. For example, in the 1980s, monitoring for hepatitis C was in its infancy (then only a few agencies in the world tested blood for what was then termed "non-A, non-B hepatitis"). Since then, definitive tests for the hepatitis C virus at the nucleic acid level have been developed and put into routine use.

Within the past 20 years, emerging diseases such the lethal fever and tissue destruction caused by the **Ebola virus** have been important blood borne threats. These so-called **hemorrhagic fevers** may have become more prominent because of human encroachment onto formerly wild regions, particularly in Africa.

Health care workers are particularly at risk of acquiring a blood borne infection. Open wounds present an opportunity for blood to splatter on a cut or scratch of a doctor or nurse. Also, the use of needles presents a risk of accidental puncture of the skin to doctors, nurses and even to custodial workers responsible for collecting the debris of hospital care.

Another group particularly at risk of blood borne infections are hemophiliacs. The necessity of hemophiliacs to receive blood products that promote clotting leaves them vulnerable. For example, in the United States, some 20% of adult hemophiliacs are infected with HIV, about 56% are infected with the hepatitis B virus, and almost 90% are infected with the hepatitis C virus. HIV is the most common cause of death among hemophiliacs.

Other viruses pose a potential for blood borne transmission. Human herpesvirus 6 and 7, **Epstein-Barr virus** and cytomegalovirus require close contact between mucous membranes for person-to-person transfer. Abrasions in the genital area may allow for the transfer of the viruses in the blood. Parvovirus, which causes the rash known as fifth disease in children, can be transferred between adults in the blood. In adults, particularly women, the resulting infection can cause arthritis

At least in North America, the increasing urbanization is bringing people into closer contact with wildlife. This has resulted in an increase in the incidence of certain blood borne diseases that are transmitted by ticks. Mice, chipmunks, and deer are two reservoirs of *Borrelia burgdorferi*, the bacterium that causes **Lyme disease**. The increasing deer population over the past 35 years in the state of Connecticut has paralleled the increasing number of cases of Lyme disease, over 3,000 in 1996 alone.

Other blood borne disease transmitted by ticks includes Rocky Mountain Spotted Fever, human granulolytic ehrlichiosis, and babesiosis. While these diseases can ultimately affect various sites in the body, their origin is in the blood.

The institution of improved means of monitoring donated blood and blood products has lowered the number of cases of blood borne infections. However, similar success in the hospital or natural settings has not occurred, and likely will not. Avoidance of infected people and the wearing of appropriate garments (such as socks and long pants when walking in forested areas where ticks may be present) are the best strategies to avoid such blood borne infections at the present time.

See also AIDS; Hemorrhagic fevers and diseases; Transmission of pathogens

BLUE-GREEN ALGAE

Blue-green algae are actually a type of **bacteria** that is known as cyanobacteria. In their aquatic habitat, cyanobacteria are equipped to use the sun's energy to manufacture their own food through **photosynthesis**. The moniker blue-green algae came about because of the color, which was a by-product of the photosynthetic activity of the microbes, and their discovery as a algal-like scum on the surface of ponds. They were assumed to be algae until their identity as bacteria was determined.

Although the recognition of the bacterial nature of the microbe occurred recently, cyanobacteria are ancient. Fossils of cyanobacteria have been found that date back 3.5 billion years and are among the oldest fossils of any life from thus far discovered on Earth. These **microorganisms** must have developed very early following the establishment of land on Earth, because the oldest known rocks are only slightly older at 3.8 billion years.

Modern day examples of cyanobacteria include *Nostoc*, *Oscillatoria*, *Spirulina*, *Microcystis*, and *Anabaena*

Cyanobacteria were monumentally important in shaping life on this planet. The oxygen atmosphere that supports human and other life was generated by cyanobacterial activity in the distant past. Many oil deposits that were laid down in the Proterozoic Era were due the activity of cyanobacteria. Another huge contribution of cyanobacteria is their role in the genesis of plants. The plant organelle known as a **chloroplast**, which the plant uses to manufacture food, is a remnant of a cyanobacterium that took up residence in a eukaryotic cell sometime in the Proterozoic or early Cambrian Era. The mitochondrion in eukaryotic cells also arose in this fashion.

The ability of cyanobacteria to photosynthetically utilize sunlight as an energy source is due to a pigment called phycocyanin. The microbes also contain the same **chlorophyll** a compound used by plants. Some blue-green algae possess a different photosynthetic pigment, which is known as phycoerythrin. This pigment imparts a red or pink color to the cells. An example is *Spirulina*. The pink color of African flamingos actually results, in part, from their ingestion of *Spirulina*.

Cyanobacteria tend to proliferate in very slow moving or still fresh water. Large populations can result very quickly, given the appropriate conditions of temperature and nutrient availability. This explosive growth is popularly referred to as a bloom. Accounts of blooms attributable to cyanobacteria date back to the twelfth century. The toxic capabilities of the organism have been known for over 100 years. Some species produce a toxin that can be released into the water upon the death of the microorganism. One of the cyanobacterial toxins is damaging to the liver, and so is designated a hepatotoxin. Another cyanobacterial toxin is damaging to cells of the nervous system, and so is a neurotoxin. Still other cyanobacterial toxins cause skin irritation.

A toxin of particular note is called microcystin. This toxin is produced by *Microcystis aeruginosa*. The microcystin toxin is the most common in water, likely because of its stability in this environment. One type of microcystin, which is designated microcystin-LR, is found in waters all over the world, and is a common cause of cyanobacterial poisoning of humans and animals.

At low levels, toxins such as microcystin produce more of an uncomfortable feeling than actual damage to the body. However, blue-green algae and their toxins can become concentrated in shallow, slow-moving bodies of water or in fish. Ingestion of the fish or accidental swallowing of the water while swimming can produce nausea, vomiting fever, and diarrhea. Eyes can also become irritated. These symptoms can be more exacerbated in children, because the toxin-to-body-weight ratio is higher in children than in adults. Liver damage can result in children exposed to the toxins.

In contrast to many other toxins, the cyanobacterial toxins can still remain potent after toxin-contaminated water has been boiled. Only the complete removal of the toxin from the water is an assurance of safety. Some success in the removal

of toxins has been claimed by the use of charcoal and by techniques that oxidize the water.

Cyanobacteria are one of the few microorganisms that can convert inert atmospheric nitrogen into a usable form, such as nitrate or ammonia. For example, the cyanobacterium *Anabaena* co-exist with a type of fern called *Azolla*, where it supplies nitrogen to the plant. The production of rice has benefited from the fertilization capability of this bacterial-plant association. The cyanobacterium *Spirulina* is a popular, high protein food source.

See also Fossilization of bacteria; Photosynthetic microorganisms

BORDATELLA PERTUSSIS • *see* PERTUSSIS

BORDET, JULES (1870-1961)

Belgian physician

Jules Bordet's pioneering research made clear the exact manner by which serums and antiserums act to destroy **bacteria** and foreign blood cells in the body, thus explaining how human and animal bodies defend themselves against the invasion of foreign elements. Bordet was also responsible for developing **complement** fixation tests, which made possible the early detection of many disease-causing bacteria in human and animal blood. For his various discoveries in the field of **immunology**, Bordet was awarded the Nobel Prize for medicine or physiology in 1919.

Jules Jean Baptiste Vincent Bordet was born in Soignies, Belgium, a small town situated twenty-three miles southwest of Brussels. He was the second son of Charles Bordet, a schoolteacher, and Célestine Vandenabeele Bordet. The family moved to Brussels in 1874, when his father received an appointment to the École Moyenne, a primary school. Jules and his older brother Charles attended this school and then received their secondary education at the Athéné Royal of Brussels. It was at this time that Bordet became interested in chemistry and began working in a small laboratory that he constructed at home. He entered the medical program at the Free University of Brussels at the age of sixteen, receiving his doctorate of medicine in 1892. Bordet began his research career while still in medical school, and in 1892 published a paper on the adaptation of **viruses** to vaccinated organisms in the *Annales de l'Institut Pasteur* of Paris. For this work, the Belgian government awarded him a scholarship to the Pasteur Institute, and from 1894 to 1901, Bordet stayed in Paris at the laboratory of the Ukrainian-born scientist **Élie Metchnikoff**. In 1899, Bordet married Marthe Levoz; they eventually had two daughters, and a son who also became a medical scientist.

During his seven years at the Pasteur Institute, Bordet made most of the basic discoveries that led to his Nobel Prize of 1919. Soon after his arrival at the Institute, he began work on a problem in immunology. In 1894, Richard Pfeiffer, a German scientist, had discovered that when cholera bacteria was injected into the peritoneum of a guinea pig immunized against the infection, the pig would rapidly die. This bacteriolysis, Bordet discovered, did not occur when the bacteria was injected into a non-immunized guinea pig, but did so when the same animal received the **antiserum** from an immunized animal. Moreover, the bacteriolysis did not take place when the bacteria and the antiserum were mixed in a test tube unless fresh antiserum was used. However, when Bordet heated the antiserum to 55 degrees centigrade, it lost its power to kill bacteria. Finding that he could restore the bacteriolytic power of the antiserum if he added a little fresh serum from a non-immunized animal, Bordet concluded that the bacteria-killing phenomenon was due to the combined action of two distinct substances: an **antibody** in the antiserum, which specifically acted against a particular kind of bacterium; and a non-specific substance, sensitive to heat, found in all animal serums, which Bordet called "alexine" (later named "complement").

In a series of experiments conducted later, Bordet also learned that injecting red blood cells from one animal species (rabbit cells in the initial experiments) into another species (guinea pigs) caused the serum of the second species to quickly destroy the red cells of the first. And although the serum lost its power to kill the red cells when heated to 55 degrees centigrade, its potency was restored when alexine (or complement) was added. It became apparent to Bordet that hemolytic (red cell destroying) serums acted exactly as bacteriolytic serums; thus, he had uncovered the basic mechanism by which animal bodies defend or immunize themselves against the invasion of foreign elements. Eventually, Bordet and his colleagues found a way to implement their discoveries. They determined that alexine was bound or fixed to red blood cells or to bacteria during the immunizing process. When red cells were added to a normal serum mixed with a specific form of bacteria in a test tube, the bacteria remained active while the red cells were destroyed through the fixation of alexine. However, when serum containing the antibody specific to the bacteria was destroyed, the alexine and the solution separated into a layer of clear serum overlaying the intact red cells. Hence, it was possible to visually determine the presence of bacteria in a patient's blood serum. This process became known as a complement fixation test. Bordet and his associates applied these findings to various other infections, like **typhoid fever**, carbuncle, and hog cholera. **August von Wasserman** eventually used a form of the test (later known as the **Wasserman test**) to determine the presence of **syphilis** bacteria in the human blood.

Already famous by the age of thirty-one, Bordet accepted the directorship of the newly created Anti-rabies and Bacteriological Institute in Brussels in 1901; two years later, the organization was renamed the Pasteur Institute of Brussels. From 1901, Bordet was obliged to divide his time between his research and the administration of the Institute. In 1907, he also began teaching following his appointment as professor of bacteriology in the faculty of medicine at the Free University of Brussels, a position that he held until 1935. Despite his other activities, he continued his research in immunology and bacteriology. In 1906, Bordet and Octave Gengou succeeded

in isolating the bacillus that causes **pertussis** (whooping cough) in children and later developed a **vaccine** against the disease. Between 1901 and 1920, Bordet conducted important studies on the coagulation of blood. When research became impossible because of the German occupation of Belgium during World War I, Bordet devoted himself to the writing of *Traité de l'immunité dans les maladies infectieuses* (1920), a classic book in the field of immunology. He was in the United States to raise money for new medical facilities for the war-damaged Free University of Brussels when he received word that he had been awarded the Nobel Prize. After 1920, he became interested in **bacteriophage**, the family of viruses that kill many types of bacteria, publishing several articles on the subject. In 1940, Bordet retired from the directorship of the Pasteur Institute of Brussels and was succeeded by his son, Paul. Bordet himself continued to take an active interest in the work of the Institute despite his failing eyesight and a second German occupation of Belgium during World War II. Many scientists, friends, and former students gathered in a celebration of his eightieth birthday at the great hall of the Free University of Brussels in 1950. He died in Brussels in 1961.

See also Antibody and antigen; B cells or B lymphocytes; Bacteria and bacterial infection; Bacteriophage and bacteriophage typing; Blood agar, hemolysis, and hemolytic reactions; Immune system; Immunity; Immunization; T cells or T lymphocytes

BOREL, JEAN-FRANÇOIS (1933-)

Belgian immunologist

Jean-François Borel is one of the discoverers of cyclosporin. The compound is naturally produced by a variety of fungus, where is acts as an antibiotic to suppress **bacterial growth**. Borel's research in the late 1970s demonstrated that in addition to the antibiotic activity, cyclosporin could act as an immunosupressant. This latter property of the compound has been exploited in limiting the rejection of transplanted organs in humans.

Borel was born in Antwerp, Belgium. After undergraduate studies in that city, he studied at the Swiss Federal Institute of Technology in Zurich. He obtained his Ph.D. in immunogenetics 1964. From there he obtained training in veterinary immunogenetics. In 1965, he moved to the Swiss Research Institute Department of Medicine where he studied **immunology**, particularly the inflammatory response. Five years later, he joined the scientific staff at Sandoz (now Novartis). He has been director of the immunology and microbiology departments at this company. Since 1983, Borel has been Vice-President of the Pharma division of Novartis. Since 1981, Borel has also been a professor of immunopharmacology in the medical faculty at the University of Bern.

In 1971, Borel isolated a compound (subsequently called cyclosporin) from a sample of the fungus *Beauvaria nivea* that was obtained during a hike by a Sandoz employee who had vacationed in the United States. Analyses by Borel showed that, unlike other immunosupressants then known, the isolated compound selectively suppressed the **T cells** of the **immune system**. The compound was obtained in pure from in 1973. By the end of that decade, Borel had demonstrated the antirejection powers of the drug in humans.

During this period, Borel is remembered for having tested the putative immunosupressant drug on himself. The compound was found to be insoluble. When Borel dissolved some of the compound in alcohol (subsequently, the use of olive oil as an emulsifier proved more efficient) and drank it, the compound subsequently appeared in his blood. This was a major finding, indicating that the compound might be amenable to injection so as to control the immune rejection of transplanted organs.

There has been a controversy as to whether Borel or another Sandoz scientist (Harold Stähelin) was primarily responsible for the discovery of cyclosporin. Both were actively involved at various stages in the purification and testing of the compound, and the primary contribution is difficult to assign. Nonetheless, it was Borel who first established the immunosuppressant effect of cyclosporin, during routine testing of compounds isolated from **fungi** for antibiotic activity.

Beginning in the 1980s, cyclosporin was licensed for use in transplantations. Since then, hundreds of thousands of people have successfully received organ transplants, where none would have before the discovery of cyclosporin.

The research of Borel and his colleagues inspired the search for other immunosupressant therapies. In recognition of his fundamental achievement to the advancement of organ transplantation, Borel received the prestigious Gairdner Award in 1986.

See also Antibody and antigen; Immunosuppressant drugs

BORRELIA BURGDORFERI • *see* LYME DISEASE

BOTULISM

Botulism is an illness produced by a toxin that is released by the soil bacterium *Clostridium botulinum*. One type of toxin is also produced by *Clostridium baratii*. The toxins affect nerves and can produce paralysis. The paralysis can affect the functioning of organs and tissues that are vital to life.

There are three main kinds of botulism. The first is conveyed by food containing the botulism toxin. Contaminated food can produce the illness after being ingested. Growth of the **bacteria** in the food may occur, but is not necessary for botulism. Just the presence of the toxin is sufficient. Thus, this form of botulism is a food intoxication (as compared with food poisoning, where **bacterial growth** is necessary). The second way that botulism can be produced is via infection of an open wound with *Clostridium botulinum*. Growth of the bacteria in the wound leads to the production of the toxin, which can diffuse into the bloodstream. The wound mode of toxin entry is commonly found in intravenous drug abusers. Finally, botulism can occur in young children following the consumption

of the organism, typically when hands dirty from outdoor play are put into the mouth.

The latter means of acquiring botulism involves the form of the bacterium known as a spore. A spore is a biologically dormant but environmentally resilient casing around the bacterium's genetic material. The spore form allows the organism to survive through prolonged periods of inhospitable conditions. When conditions improve, such as when a spore in soil is ingested, resuscitation, growth of the bacterium, and toxin production can resume. For example, foodborne botulism is associated with canned foods where the food was not heated sufficiently prior to canning to kill the spores.

Botulism is relatively rare. In the United States, just over 100 cases are reported each year, on average. The number of cases of foodborne and infant botulism has not changed appreciably through the 1990s to the present day. Foodborne cases have tended to involve the improper preparation of home-canned foods.

There are seven known types of botulism toxin, based on their antigenic make-up. These are designated toxins A through G. Of these, only types A, B, E, and F typically cause botulism in humans, although involvement of type C toxin in infants has been reported, and may be particularly associated with the consumption of contaminated honey.

Infant botulism caused by toxin type C may be different from the other types of botulism in that the toxin is produced in the person following the ingestion of living *Clostridium botulinum*.

The toxins share similarities in their gross structure and in their mechanism of action. The toxins act by binding to the region of nerve cells that is involved in the release of a chemical known as a neurotransmitter. Neurotransmitters travel across the gap (synapse) separating neurons (nerve cells) and are essential to the continued propagation of a neural impulse. Accordingly, they are vital in maintaining the flow of a transmitted signal from nerve to nerve. Blocking nerve transmissions inhibits the means by which the body can initiate the movement of muscles. The result is paralysis. This paralysis produces a variety of symptoms including double or blurred vision, drooping eyelids, slurred speech, difficulties in swallowing, muscle weakness, paralysis of limbs and respiratory muscles.

The appearance of the symptoms of botulism vary depending on the route of toxin entry. For example, ingestion of toxin-contaminated food usually leads to symptoms within two to three days. However, symptoms can appear sooner or later depending on whether the quantity of toxin ingested is low or high.

The diagnosis of botulism and so the start of the appropriate therapy can be delayed, due to the relative infrequency of the malady and its similarity (in the early stages) with other maladies, such as Guillain-Barré syndrome and stroke. Diagnosis can involve the detection of toxin in the patient's serum, isolation of living bacteria from the feces, or by the ability of the patient's sample to produce botulism when introduced into test animals.

Clostridium botulinum requires an oxygen-free atmosphere to grow. Growth of the bacteria is associated with the production of gas. Thus, canned foods can display a bulging lid, due to the build-up of internal pressure. Recognition of this phenomenon and discarding of the unopened can is always a safe preventative measure.

Studies conducted by United States health authorities have shown that the different forms of the botulism toxin display some differences in their symptomatology and geographic distribution. Type A associated botulism is most prevalent in the western regions of the US, particularly in the Rocky Mountains. This toxin produces the most severe and long-lasting paralysis. Type B toxin is more common in the eastern regions of the country, especially in the Allegheny mountain range. The paralysis produced by type B toxin is less severe than with type A toxin. Type E botulism toxin is found more in the sediments of fresh water bodies, such as the Great Lakes. Finally, type F is distinctive as it is produced by *Clostridium baratii*.

Treatment for botulism often involves the administration of an antitoxin, which acts to block the binding of the toxin to the nerve cells. With time, paralysis fades. However, recovery can take a long time. If botulism is suspected soon after exposure to the bacteria, the stomach contents can be pumped out to remove the toxic bacteria, or the wound can be cleaned and disinfected. In cases of respiratory involvement, the patient may need mechanical assistance with breathing until lung function is restored. These measures have reduced the death rate from botulism to 8% from 50% over the past half century.

As dangerous as botulinum toxin is when ingested or when present in the bloodstream, the use of the toxin has been a boon to those seeking non-surgical removal of wrinkles. Intramuscular injection of the so-called "Botox" relaxes muscles and so relieves wrinkles. Thus far, no ill effects of the cosmetic enhancement have appeared. As well, Botox may offer relief to those suffering from the spastic muscle contractions that are a hallmark of cerebral palsy.

See also Bacteria and bacterial diseases; Bioterrorism; Food safety

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

• *see* BSE AND CJD DISEASE

BOYER, HERBERT WAYNE (1936-)

American molecular geneticist

In 1973 Herbert Boyer was part of the scientific team that first described the complete process of **gene** splicing, which is a basic technique of genetic engineering (recombinant **DNA**). Gene splicing involves isolating DNA, cutting out a piece of it at known locations with an enzyme, then inserting the fragment into another individual's genetic material, where it functions normally.

Boyer was born in Pittsburgh and received a bachelor's degree in 1958 from St. Vincent College. At the University of

Pittsburgh he earned an M.S. in 1960 and a Ph.D. in bacteriology in 1963. In 1966 Boyer joined the **biochemistry** and biophysics faculty at the University of California, San Francisco, where he continues his research.

Boyer performed his work with Stanley Cohen from the Stanford School of Medicine and other colleagues from both Stanford and the University of California, San Francisco. The scientists began by isolating a plasmid (circular DNA) from the **bacteria** *E. coli* that contains genes for an **antibiotic resistance** factor. They next constructed a new plasmid in the laboratory by cutting that plasmid with restriction endonucleases (**enzymes**) and joining it with fragments of other **plasmids**.

After inserting the engineered plasmid into *E. coli* bacteria, the scientists demonstrated that it possessed the DNA nucleotide sequences and genetic functions of both original plasmid fragments. They recognized that the method allowed bacterial **plasmids** to replicate even though sequences from completely different types of cells had been spliced into them.

Boyer and his colleagues demonstrated this by **cloning** DNA from one bacteria species to another and also cloning animal genes in *E. coli*.

Boyer is a co-founder of the genetic engineering firm Genentech, Inc. and a member National Academy of Sciences. His many honors include the Albert and Mary Lasker Basic Medical Research Award in 1980, the National Medal of Technology in 1989, and the National Medal of Science in 1990.

See also Molecular biology and molecular genetics

BRENNER, SYDNEY (1927-)

South African–English molecular biologist

Sydney Brenner is a geneticist and molecular biologist who has worked in the laboratories of Cambridge University since 1957. Brenner played an integral part in the discovery and understanding of the triplet **genetic code** of **DNA**. He was also a member of the first scientific team to introduce messenger **RNA**, helping to explain the mechanism by which genetic information is transferred from DNA to the production of **proteins and enzymes**. In later years, Brenner conducted a massive, award-winning research project, diagramming the nervous system of a particular species of worm and attempting to map its entire genome.

Brenner was born in Germiston, South Africa. His parents were neither British nor South African—Morris Brenner was a Lithuanian exile who worked as a cobbler, and Lena Blacher Brenner was a Russian immigrant. Sydney Brenner grew up in his native town, attending Germiston High School. At the age of fifteen, he won an academic scholarship to the University of the Witwatersrand in Johannesburg, where he earned a master's degree in medical biology in 1947. In 1951, Brenner received his bachelor's degree in medicine, the qualifying degree for practicing physicians in Britain and many of its colonies. The South African university system could offer him no further education, so he embarked on independent research. Brenner studied **chromosomes**, cell structure, and staining techniques, built his own centrifuge, and laid the foundation for his interest in **molecular biology**.

Frustrated by lack of resources and eager to pursue his interest in molecular biology, Brenner decided to seek education elsewhere, and was encouraged by colleagues to contact Cyril Hinshelwood, professor of physical chemistry at Oxford University. In 1952, Hinshelwood accepted Brenner as a doctoral candidate and put him to work studying a **bacteriophage**, a virus that had become the organism of choice for studying molecular biology in living systems. Brenner's change of location was an important boost to his career; while at Oxford he met Seymour Benzer, with whom Brenner collaborated on important research into **gene** mapping, sequencing, **mutations** and colinearity. He also met and exchanged ideas with **James Watson** and **Francis Crick**, the Cambridge duo who published the first paper elucidating the structure of DNA, or **deoxyribonucleic acid**, the basic genetic molecule. Brenner and Crick were to become the two most important figures in determining the general nature of the genetic code.

Brenner earned his Ph.D. from Oxford in 1954, while still involved in breakthrough research in molecular biology. His colleagues tried to find a job for him in England, but he accepted a position as lecturer in physiology at the University of the Witwatersrand and returned to South Africa in 1955. Brenner immediately set up a laboratory in Johannesburg to continue his phage research, but missed the resources he had enjoyed while in England. Enduring almost three years of isolation, Brenner maintained contact with his colleagues by mail.

In January 1957, Brenner was appointed to the staff of the Medical Research Council's Laboratory of Molecular Biology at Cambridge, and he and his family were able to settle in England permanently. Brenner immediately attended to theoretical research on the characteristics of the genetic code that he had begun in Johannesburg, despite the chaotic atmosphere. At the time, the world's foremost geneticists and molecular biologists were debating about the manner in which the sequences of DNA's four nucleotide bases were interpreted by an organism. The structure of a DNA molecule is a long, two-stranded chain that resembles a twisted ladder. The sides of the ladder are formed by alternating phosphate and sugar groups. The nucleotide bases adenine, guanine, thymine, and cytosine—or A, G, T, and C—form the rungs, a single base anchored to a sugar on one side of the ladder and linked by hydrogen bonds to a base similarly anchored on the other side. Adenine bonds only with thymine and guanine only with cytosine, and this complementarity is what makes it possible to replicate DNA. Most believed that the bases down the rungs of the ladder were read three at a time, in triplets such as ACG, CAA, and so forth. These triplets were also called codons, a term coined by Brenner. Each codon represented an amino acid, and the amino acids were strung together to construct a protein. The problem was in understanding how the body knew where to start reading; for example, the sequence AACCGGTT could be read in several sets of three-letter sequences. If the code were overlapping, it could be read AAC, ACC, CCG, and so forth.

Brenner's contribution was his simple theoretical proof that the base triplets must be read one after another and could

not overlap. He demonstrated that an overlapping code would put serious restrictions on the possible sequences of amino acids. For example, in an overlapping code the triplet AAA, coding for a particular amino acid, could only be followed by an amino acid coded by a triplet beginning with AA—AAT, AAA, AAG, or AAC. After exploring the amino acid sequences present in naturally occurring proteins, Brenner concluded that the sequences were not subject to these restrictions, eliminating the possibility of an overlapping code. In 1961, Brenner, in collaboration with Francis Crick and others, confirmed his theory with bacteriophage research, demonstrating that the construction of a bacteriophage's protein coat could be halted by a single "nonsense" mutation in the organism's genetic code, and the length of the coat when the **transcription** stopped corresponded to the location of the mutation. Interestingly, Brenner's original proof was written before scientists had even determined the universal genetic code, although it opened the door for sequencing research.

Also in 1961, working with Crick, **François Jacob**, and **Matthew Meselson,** Brenner made his best-known contribution to molecular biology, the discovery of the messenger RNA (mRNA). Biologists knew that DNA, which is located in the **nucleus** of the cell, contains a code that controlled the production of protein. They also knew that protein is produced in structures called **ribosomes** in the cell **cytoplasm**, but did not know how the DNA's message is transmitted to, or received by, the ribosomes. RNA had been found within the ribosomes, but did not seem to relate to the DNA in an interesting way. Brenner's team, through original research and also by clever interpretation of the work of others, discovered a different type of RNA, mRNA, which was constructed in the nucleus as a template for a specific gene, and was then transported to the ribosomes for transcription. The RNA found within the ribosomes, rRNA, was only involved in the construction of proteins, not the coding of them. The ribosomes were like protein factories, following the instructions delivered to them by the messenger RNA. This was a landmark discovery in genetics and cell biology for which Brenner earned several honors, including the Albert Lasker Medical Research Award in 1971, one of America's most prestigious scientific awards.

In 1963 Brenner set out to expand the scope of his research. For most of his career, he had concentrated on the most fundamental chemical processes of life, and now he wanted to explore how those processes governed development and regulation within a living organism. He chose the nematode *Caenorhabditis elegans,* a worm no more than a millimeter long. As reported in *Science,* Brenner had initially told colleagues, "I would like to tame a small metazoan," expecting that the simple worm would be understood after a small bit of research. As it turned out, the nematode project was to span three decades, involve almost one hundred laboratories and countless researchers, make *C. elegans* one of the world's most studied and best understood organisms, and become one of the most important research projects in the history of genetics.

Brenner's nematode was an ideal subject because it was transparent, allowing scientists to observe every cell in its body, and had a life cycle of only three days. Brenner and his assistants observed thousands of *C. elegans* through every stage of development, gathering enough data to actually trace the lineage of each of its 959 somatic cells from a single zygote. Brenner's team also mapped the worm's entire nervous system by examining electron micrographs and producing a wiring diagram that showed all the connections among all of the 309 neurons. This breakthrough research led Brenner to new discoveries concerning sex determination, brain chemistry, and programmed cell death. Brenner also investigated the genome of the nematode, a project that eventually led to another milestone, a physical map of virtually the entire genetic content of *C. elegans*. This physical map enabled researchers to find a specific gene not by initiating hundreds of painstaking experiments, but by reaching into the freezer and pulling out the part of the DNA that they desired. In fact, Brenner's team was able to distribute copies of the physical map, handing out the worm's entire genome on a postcard-size piece of filter paper.

Brenner's ultimate objective was to understand development and behavior in genetic terms. He originally sought a chemical relationship that would explain how the simple molecular mechanisms he had previously studied might control the process of development. As his research progressed, however, he discovered that development was not a logical, program-driven process—it involved a complex network of organizational principles. Brenner's worm project was his attempt to understand the next level in the hierarchy of development. What he and his assistants have learned from *C. elegans* may have broad implications about the limits and difficulties of understanding behavior through gene sequencing. The Human Genome Project, for instance, was a mammoth effort to sequence the entire human DNA. James Watson has pointed to Brenner's worm experiments as a model for the project.

Brenner's research has earned him worldwide admiration. He has received numerous international awards, including the 1970 Gregor Mendel Medal from the German Academy of Sciences, the prestigious Kyoto Prize from Japan, as well as honors from France, Switzerland, Israel, and the United States. He has been awarded honorary degrees from several institutions, including Oxford and the University of Chicago, and has taught at Princeton, Harvard, and Glasgow Universities. Brenner is known for his aggressiveness, intelligence, flamboyance, and wit. His tendency to engage in remarkably ambitious projects such as the nematode project, as well as his ability to derive landmark discoveries from them, led *Nature* to claim that Brenner is "alternatively molecular biology's favorite son and *enfant terrible*."

While still in Johannesburg in 1952, Brenner married May Woolf Balkind. He has two daughters, one son, and one stepson. In 1986, the Medical Research Council at Cambridge set up a new **molecular genetics** unit, and appointed Brenner to a lifelong term as its head. Research at the new unit is centered on Brenner's previous work on *C. elegans* and the mapping and **evolution** of genes.

See also Bacteriophage and bacteriophage typing; Genetic code; Genetic identification of microorganisms; Genetic mapping; Microbial genetics

BROCK, THOMAS D. (1926-)

American bacteriologist

Thomas D. Brock was born in Cleveland, Ohio, and has lived in the midwestern states of the United States all his life. Brock's 1967 summary article in *Science,* entitled "Life at High Temperatures" generated a great deal of interest, and spawned the branch of microbiology concerned with **bacteria** that live in extreme environments.

After graduating from high school in Chillicothe, Ohio, Brock enlisted in the Navy. As a veteran, he enrolled at Ohio State University in 1946. He graduated with a degree in botany in 1949, a MS degree and a Ph.D. in 1952. After graduation he joined the **antibiotics** research department at the Upjohn Company. His relative lack of microbiology training to that point necessitated that he learn on the job. This embracing of new aspects of research continued throughout his microbiology career. Leaving Upjohn after five years, he accepted a position at Western Reserve University (now Case Western University). In 1960 he moved to Indiana University as an Assistant Professor of Bacteriology. He remained there until 1971.

In 1963, Brock had the opportunity to pursue **marine microbiology** research at the Friday Harbor Laboratories of the University of Washington. There he studied *Leucothrix mucor*. His diagrams of the twisted configurations called "knots" formed by the growing organisms became a cover story in *Science* and were featured in the *New York Times*. This work also stimulated his interest in the microbial ecology of sulfur springs, which led him to conduct research at Yellowstone National Park over the next decade.

Beginning in the mid 1960s, Brock began field research in Yellowstone National Park, Montana. At the time of these studies, bacterial life was not thought to be possible at growth temperatures above about 80° C. Brock found **microorganisms** that were capable of growth and division at temperatures of nearly 100° C, the temperature at which water boils.

In particular, Brock isolated and named the bacterium *Thermus aquaticus*. This microbe was the first so-called archaebacteria to be discovered. Archaebacteria are now known to be a very ancient form of life, and may even constitute a separate kingdom of life. The discovery of *Thermus aquaticus* is thus, one of the fundamental milestones of microbiology.

Brock's discovery has also had a significant impact in the field of **biotechnology**. The **enzymes** of the bacterium are designed to work at high temperatures. In particular, a polymerase is the basis of the **polymerase chain reaction** that is used to artificially amplify the amount of **deoxyribonucleic acid**. The use of **PCR** has spawned a multi-billion dollar biotechnology industry.

In 1971, Brock moved to the Department of Bacteriology at the University of Wisconsin-Madison. He is currently E.B. Fred Professor of Natural Sciences-Emeritus at Wisconsin.

Brock has also been a prolific writer and scientific historian. His numerous books include volumes on the biology of microorganisms, the principles of microbial ecology, the milestones in microbiology, and a profile of **Robert Koch**. In the 1980s, he formed his own scientific publishing company, which continues to the present day.

For his groundbreaking research and publishing efforts, Brock has received many scientific achievement and education awards in the United States and worldwide.

See also Extremophiles; Tag enzyme

BROTH • *see* GROWTH AND GROWTH MEDIA

BROWNIAN MOTION • *see* BACTERIAL MOVEMENT

BRUCELLOSIS

Brucellosis is a disease caused by **bacteria** in the genus Brucella. The disease infects animals such as swine, cattle, and sheep; humans can become infected indirectly through contact with infected animals or by drinking Brucella-contaminated milk. In the United States, most domestic animals are vaccinated against the bacteria, but brucellosis remains a risk with imported animal products.

Brucella are rod-shaped bacteria that lack a capsule around their cell membranes. Unlike most bacteria, Brucella cause infection by actually entering host cells. As the bacteria cross the host cell membrane, they are engulfed by host cell vacuoles called phagosomes. The presence of Brucella within host cell phagosomes initiates a characteristic immune response, in which infected cells begin to stick together and form aggregations called granulomas.

Three species of Brucella cause brucellosis in humans: *Brucella melitensis,* which infects goats; *B. abortis,* which infects cattle and, if the animal is pregnant, causes the spontaneous abortion of the fetus; and *B. suis,* which infects pigs. In animals, brucellosis is a self-limiting disease, and usually no treatment is necessary for the resolution of the disease. However, for a period of time from a few days to several weeks, infected animals may continue to excrete brucella into their urine and milk. Under warm, moist conditions, the bacteria may survive for months in soil, milk, and even seawater.

Because the bacteria are so hardy, humans may become infected with Brucella by direct contact with the bacteria. Handling or cleaning up after infected animals may put a person in contact with the bacteria. Brucella are extremely efficient in crossing the human skin barrier through cuts or breaks in the skin.

The incubation period of Brucella, the time from exposure to the bacteria to the start of symptoms, is typically about three weeks. The primary complaints are weakness and fatigue. An infected person may also experience muscle aches, fever, and chills.

The course of the disease reflects the location of the Brucella bacteria within the human host. Soon after the Brucella are introduced into the bloodstream, the bacteria seek out the nearest lymph nodes and invade the lymph node cells.

From the initial lymph node, the Brucella spread out to other organ targets, including the spleen, bone marrow, and liver. Inside these organs, the infected cells form granulomas.

Diagnosing brucellosis involves culturing the blood, liver, or bone marrow for Brucella organisms. A positive **culture** alone does not signify brucellosis, since persons who have been treated for the disease may continue to harbor Brucella bacteria for several months. Confirmation of brucellosis, therefore, includes a culture positive for Brucella bacteria as well as evidence of the characteristic symptoms and a history of possible contact with infected milk or other animal products.

In humans, brucellosis caused by *B. abortus* is a mild disease that resolves itself without treatment. Brucellosis caused by *B. melitensis* and *B. suis,* however, can be chronic and severe. Brucellosis is treated with administration of an antibiotic that penetrates host cells to destroy the invasive bacteria.

Since the invention of an animal **vaccine** for brucellosis in the 1970s, the disease has become somewhat rare in the United States. Yet the vaccine cannot prevent all incidence of brucellosis. The **Centers for Disease Control** usually reports fewer than 100 total cases per year in the United States. Most of these were reported in persons who worked in the meat processing industry. Brucellosis remains a risk for those who work in close contact with animals, including veterinarians, farmers, and dairy workers.

Brucellosis also remains a risk when animal products from foreign countries are imported into the United States. Outbreaks of brucellosis have been linked to unpasteurized feta and goat cheeses from the Mediterranean region and Europe. In the 1960s, brucellosis was linked to bongo drums imported from Africa; drums made with infected animal skins can harbor Brucella bacteria, which can be transmitted to humans through cuts and scrapes in the human skin surface.

In the United States, preventive measures include a rigorous **vaccination** program that involves all animals in the meat processing industry. On an individual level, people can avoid the disease by not eating animal products imported from countries where brucellosis is frequent, and by avoiding foods made with unpasteurized milk.

See also Bacteria and bacterial infection; Food safety; Infection and resistance; Pasteurization

BSE AND CJD DISEASE

Bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob Disease (CJD) are ailments in which the functioning of the brain is progressively impaired. Both diseases are associated with visually abnormal pinpoints (or plaques) in the brain, and in a changed texture of the brain tissue. The brain tissue, particularly in the cortex and cerebellum, becomes filled with large open spaces (vacuoles) and becomes spongy in texture. The "spongiform" part of BSE comes from this texture characteristic.

BSE is a disease of animals such as cattle and sheep, while CJD is associated with humans. However, the two diseases may have a common cause. The cause of BSE and CJD, and of other diseases such as scrapie, transmissible mink encephalopathy, fatal familial insomnia, and kuru, are **prions**. Prions are particles that are made solely of protein. Even though they lack genetic material, they are infectious.

Both BSE and CJD are characterised by a loss of coordination and the control over functions such as grasping and holding, dementia, paralysis, eventually leading to death. There is no cure for either disease, and no are vaccines available.

CJD derives its name from its discoverers. Progressive and ultimately fatal dementia that was accompanied by other neurological abnormalities was described in six patients in the 1920s by two German neuroscientists, Hans Gerhard Creutzfeldt and Alphons Maria Jakob. In the 1960s, the neurological changes associated with the development of CJD had become accepted by the medical community.

The average incidence rate for CJD over time is about one person per million. Clusters of CJD do occur. The most recent example is the 48 confirmed cases that were diagnosed in Britain between 1996 and 2001. There is no evident predilection for a gender, any ethnic group, or for geographical location. However, the incidence in those over 55 years of age is far higher (over 30 times) than for those under 55 years.

Three means of acquiring CJD have been identified. First, the disease can be genetically inherited. This is also described as familial CJD. Secondly, the disease can appear with no exact origin being known. About 85% of CJD cases are of this unknown variety. Lastly, the disease can be acquired during surgery. This so-called iatrogenic form is typically a result of CJD-contaminated equipment or tissue (brain and corneal grafts are two examples).

There is no cure for CJD. Treatment consists of managing the patient so that his/her increasingly impaired mental faculties do not result in injury, and in personal care as these functions become impossible for the person to perform themselves.

BSE causes a progressive neurological deterioration in cattle that is similar to the course of CJD in humans. Cattle with BSE are more temperamental, have problems with their posture and coordination, have progressively greater difficulty in rising off the ground and walking, produce less milk, have severe twitching of muscles, and loss weight even though their appetite is undiminished. The so-called incubation period, the time from when the animal is first infected until symptoms appear, ranges from two to eight years. After appearance of symptoms, deterioration is rapid and the animal dies or is destroyed within six months. The disease is one of several so-called transmissible spongiform encephalopathies (TSEs) in animals.

BSE was confirmed as a disease of cattle in November of 1996. Since then, almost all reported cases have been in cattle born in the United Kingdom. Other countries in Europe and Asia have reported BSE, but in far fewer numbers than in Britain. No cases have been detected in the United States (the U.S. has not imported U.K. beef since 1985 and maintains a rigorous surveillance program). As of November 2001, the total number of confirmed cases of BSE in U.K. cattle was just over 181,000. In 1993, a BSE epidemic in the U.K. peaked at almost 1,000 new cases per week. While the cause of this near-exclusivity has yet to be conclusively determined, the

2001 outbreak of hoof and mouth disease in the United Kingdom revealed that a common practice has been to feed cattle "offal," the ground up waste from the slaughter process. Experience with kuru has shown that consumption of prion-infected tissue is a means of spreading the disease.

This method of transmission is suspected of being important, if not principle, for BSE. The exact origin of the prions is not known. Sheep are considered a likely source.

Until the 1900s, scientists believed that the transmission of the BSE agent to humans did not occur. However, several studies conducted in the latter years of the 1990s has cast doubt on this assumption. Studies using mice showed that the brain injuries caused in BSE and CJD are identical. Moreover, these brain alterations occurred in mice injected with either brain tissue from BSE-diseased cattle, which was expected, or with brain tissue from CJD-diseased animals, which was unexpected. Thus, development of CJD could be due to human consumption of BSE-diseased meat.

The currently held view is that prions from cattle infected with BSE are capable of infecting humans and causing what is termed a variant CJD (vCJD) disease in humans. There is evidence that the suspect vCJD has a different infectious behavior than CJD. For instance, younger people can be infected, and the neurological symptoms differ.

The existence of a vCJD is based mainly on epidemiological evidence. If the existence of vCJD is proven, then the species barrier for the transmission of BSE and CJD does not exist. However, the possibility still remains that the contaminating agent in the meat is really a prion that causes normally CJD, and that this prion is naturally present in cattle but has escaped detection until now. If so, then BSE and CJD infections could indeed be confined to non-human and human mammals, respectively.

See also BSE and CJD disease, advances in research; BSE and CJD disease, ethical issues and socio-economic impact; Latent viruses and diseases

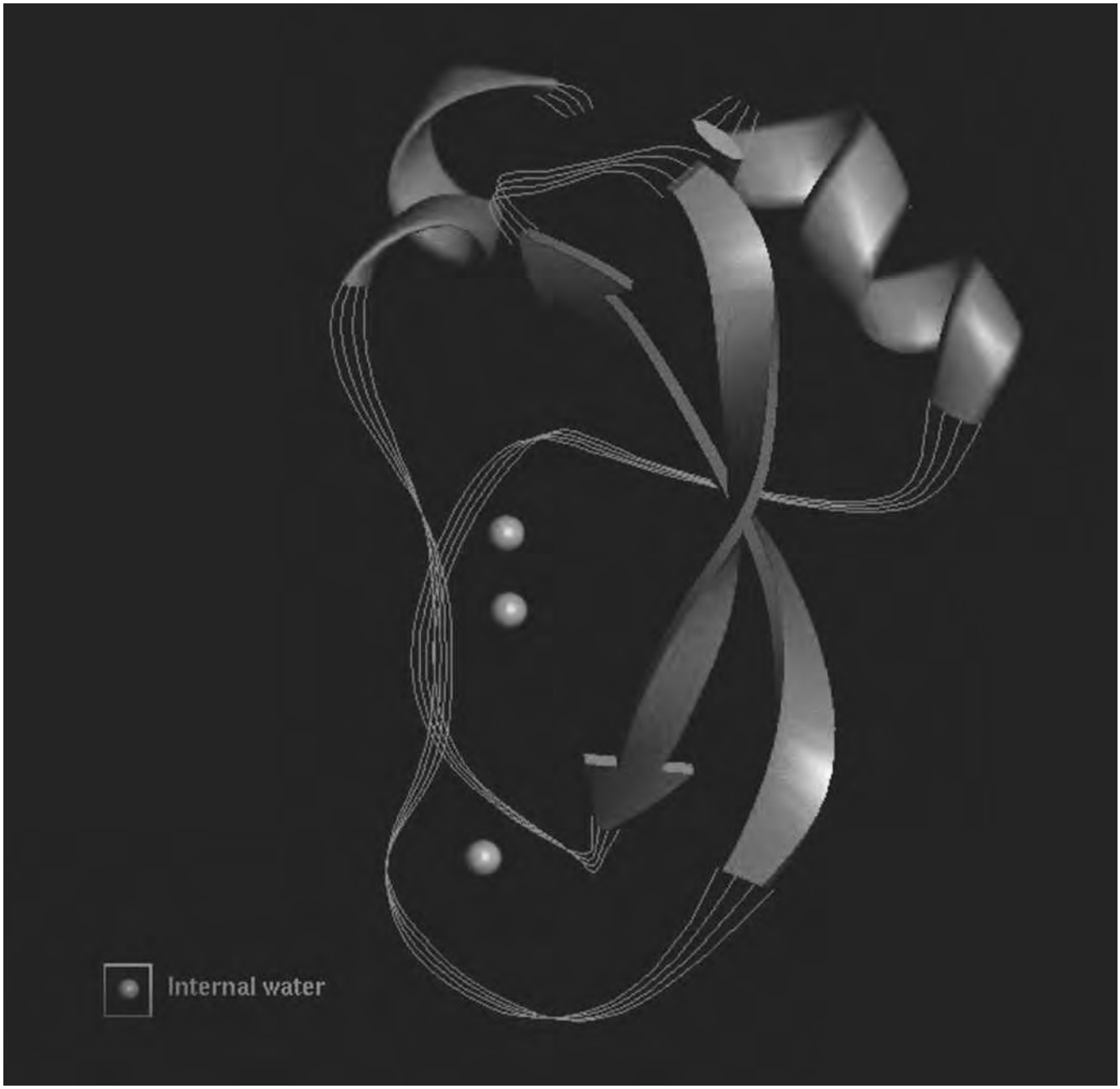
BSE AND CJD: ETHICAL ISSUES AND SOCIO-ECONOMIC IMPACT

The outbreak of bovine spongiform encephalopathy (BSE) or "mad cow disease" in the United Kingdom and continental Europe continues to concern beef and dairy producers and the general public in the United States. This concern has increased recently because of the continued spread of the disease on the European continent and the development of a similar disease that has appeared in people, mostly in the U.K. The new disease known as variant Creutzfeldt-Jakob disease (vCJD) appears to be more closely related to BSE in its pathology than to traditional CJD. It is therefore assumed that vCJD has crossed the species barrier from cattle to Man.

BSE and CJD are prion diseases, a group of rapidly progressive, fatal, untreatable neurodegenerative syndromes characterized by the accumulation in the brain of a protease-resistant protein that is the main (or only) macromolecule of the transmissible agent. The prototypical prion disease of animals is scrapie, which has been long recognized in sheep and goats as a common and economically important disorder. Following 1988, BSE has given rise to considerable economic and political turmoil in the United Kingdom as it developed in more than 150 000 cattle. At the peak of the epidemic in 1993, approximately 700 cattle were newly affected each week. The epidemic has been linked to changes in the rendering of sheep or cattle carcasses for use as protein supplements to feed-meal, suggesting that inadequately inactivated scrapie agent from sheep, cattle or both was the initial cause. Following a legislation banning the feeding of ruminant offal to livestock, the rate of diagnosed BSE has decreased. It is still uncertain whether the origins of BSE lie in a mutant form of scrapie or if it developed naturally in cattle.

A number of human prion diseases exist including CJD, kuru, Gerstmann-Sträussler-Scheinker (GSS) syndrome and fatal familial insomnia (FFI). These diseases are rare and, until recently, were not considered of any great socio-economic significance. However, in the wake of the BSE crisis in the U.K. and the suspicion that contaminated beef may lie at the root of it, many people now fear that an epidemic may be imminent. The infectivity of prion diseases appears to reside in the prion protein designated PrPSc. PrPSc is the abnormal, protease-resistant isoform of a normal cellular membrane protein designated PrPC. **Stanley B. Prusiner** of the University of California at San Francisco has long contended that changes in conformation underlie the dramatic differences in the properties of the two isoforms; by abnormal molecular folding, PrPSc acquires protease resistance and a "catalytic" ability to recruit more conformational copies of itself from PrPC. PrPSc is remarkably resistant to many procedures that inactivate conventional infectious agents and, therefore, problems have been encountered in decontamination procedures of, for example, surgical instruments. Although 90% of prion disease cases arise sporadically and a further 10% arise where the family has some history of the disease, it is an unfortunate fact that about eighty cases of CJD have arisen iatrogenically, that is, as a result of exposure to medical treatment, facilities, or personnel. Cases of transmission by corneal transplant, transplant of dura mater, exposure to infected neurosurgical instruments and electroencephalogram probes, and transplantation of human growth hormone have been confirmed.

The indestructibility of **prions** creates real problems in sterilizing surgical instruments; it is basically impossible, and equipment has retained infectivity and caused infection in patients even after repeated "sterilizations." Currently in the U.K., scientists are considering making all surgical instruments disposable. Neurosurgical equipment is already disposed of after each patient. Since vCJD is carried heavily by the lymphoreticular (blood/lymph) systems, the tonsils, appendix, and most recently, the lymph nodes of vCJD patients have been found to be full of prions, unlike in patients with classical CJD. This has brought about the worrisome debate in the U.K. that all surgical equipment, not just neurosurgical, could be contaminated and has brought on calls to destroy all surgical equipment after use or to use disposable instruments only. The U.K. has also banned the reuse of contact "fitting" lenses by



Computer model of the protein molecule responsible for plaques in the brain.

optometrists and opthamologists—hard lenses that are "sterilized" between patient use in clinics that fit contact lenses. The eye has a direct neural link with the brain and is one of the most highly infectious organs of the body in CJD and vCJD, after the brain and spinal cord. There is concern that these lenses could spread iatrogenic vCJD. Additionally "touch" tonometry has been banned in the UK, only the "air puff" method is allowed now. And, of course, ophthalmological surgery could be a prime candidate for this as well. However, nothing is being done in this regard in the U.S. to screen U.K. patients, or U.S. patients who have lived abroad.

A new fear is that blood supplies may be contaminated with prions and that Creutzfeldt-Jakob disease (CJD) will join **hepatitis** and **AIDS** in the public and medical consciousness as the next infectious disease epidemic which may be contracted through donated blood and tissue. In view of the theoretical risk of blood-borne transmission of CJD, some experts recommend that the following groups of people not donate blood: all people with CJD; first-degree relatives of CJD patients with

familial disease (determined by genetic testing, by identifying two or more first-degree relatives with CJD or, if there is no information, by a precautionary assumption of familial disease); recipients of products derived from human pituitary glands; and recipients of corneal or dura-mater grafts.

The risk of transmission of CJD through blood, blood products, and organ or tissue transplants, is being addressed by, for example, the Laboratory Centre for Disease Control (LCDC) in Canada. They are planning to initiate an enhanced surveillance system for CJD throughout Canada. Cases will be reported to the surveillance system by specialists in neurology, neuropathology and geriatrics. Through record review, interview, genetic sequencing and neuropathological examination, extensive information about every person suspected of having CJD will be collected and compared with data from a control population to ascertain the relative risk of CJD associated with exposure to blood and blood products. In addition, Canada has been invited to participate in European Concerted Action on CJD, an international surveillance program for variant CJD coordinated by investigators in Edinburgh. This obviously has ethical implications for patient privacy and it is questionable if such extreme measures are really necessary. The prion agent is not new unlike **HIV** and other emerging agents and there is an absence of any recorded cases of CJD among people with hemophilia and recipients of multiple transfusions or people who abuse injection drugs. Also, a small case-controlled study in Britain revealed no risk for the subsequent development of CJD associated with receiving blood.

See also BSE and CJD: recent advances in research; Public health, current issues

BSE, SCRAPIE, AND CJD: RECENT ADVANCES IN RESEARCH

Bovine spongiform encephalopathy (BSE) in cows, scrapie in sheep, and Creutzfeldt-Jakob disease (CJD) in humans are examples of prion diseases. The central event in the pathogenesis of these fatal disorders is hypothesized to be the post-translational conversion of a normal host protein of unknown function, termed PrPC into an abnormal isoform called PrPSc. The idea that protein alone can carry information sufficient to ensure its own propagation was an unprecedented challenge to the "central dogma" of **molecular biology** which essentially states that nucleic acids, not proteins, are the biological information carriers. The work that led to current understanding of prion diseases originated more than four decades ago. In the 1960s, Tikvah Alper and her co-workers reported that the scrapie agent was extremely resistant to treatments that normally destroy nucleic acids, but sensitive to procedures that damage proteins. Furthermore, the minimum molecular size needed in maintaining infectivity was too small to be a virus or other known infectious agent. These results led J. Griffith to propose that the material responsible for transmitting scrapie could be a protein that has the unusual ability to replicate itself in the body. Extensive work by **Stanley Prusiner** finally led to the purification of the PrP protein. For the next two decades, most research on prion diseases has focussed on the abnormal PrPSc and consequently, the functional role of PrPC has remained an enigma. Recent advances in the field of prion research, however, suggest that PrPC is a copper binding protein and has a modulating role in brain oxidative homeostasis. On the basis of *in vitro* studies by D. R. Brown at the University of Cambridge, it would appear that PrPC may act as an antioxidant enzyme in a similar manner to superoxide dismutase. The presence of the copper ion is essential for such a function.

Much recent evidence suggests that alterations in metallochemical processes could be a contributing factor for the pathological process in neurodegenerative disorders, including Alzheimer's disease and now possibly prion diseases. The PrPC protein has recently been found to have a region at its N-terminus, which is able to bind copper tightly and other metals, such as nickel, zinc and manganese, less tightly. One of the biochemical differences between the PrPC and PrPSc that was recognized very early is the surprising resistance of PrPSc to proteases (**enzymes** able to degrade proteins). *In vitro* studies have shown that if manganese ions replace the copper ions in the PrPC protein, it undergoes a structural change and becomes protease resistant. Furthermore, the binding of manganese to PrP dramatically reduces its superoxide-dismutase activity, suggesting that its cellular function may be affected under these conditions. Whether these *in vitro* changes brought about by different metal ions resemble the changes in PrP during prion disease is still to be confirmed, but the results are certainly suggestive. Research in this direction is progressing in several institutions in the UK at the moment.

High concentrations of metals are found in the brain and to prevent neuronal damage triggered by these elevated concentrations, the brain has evolved efficient mechanisms to regulate the availability of these metals. Metals are required for the normal functioning of the brain, such as the proper transmission of synaptic signals, which involve the release of zinc, copper and iron by neurons. At the same time, metals are an integral part of the cellular defense system, as they are often bound to antioxidant proteins and protect the brain from damage by free radicals. Although metals are essential to the normal functioning of the brain, perturbation in metal levels can upset cellular protein behaviour and possibly lead to neurological disorders. In Alzheimer's disease, for example, the levels of copper, zinc, and iron were found to alter in severely degenerated brain regions. In the hippocampus and amygdala regions, the levels of both zinc and iron were increased while the levels of copper were decreased.

In the 1970s, Pattison and Jebbett showed that when mice were fed with cuprizone, a copper chelator, it induced histopathological changes reminiscent of scrapie in sheep and further analysis indicated similar biochemical changes. Also a recent report by Mark Purdey showed that in the ecosystem supporting isolated clusters of sporadic prion diseases in Colorado, Iceland, and Slovakia, a consistent elevation of manganese concentration in relation to normal levels recorded in adjoining prion disease-free localities were detected. Evidence has also emerged concerning the metal content of

the brains of animals and humans with prion diseases. The most alarming finding is preliminary evidence from two independent groups that CJD patients have a ten-fold increase in the levels of manganese in their brains. This increase is unprecedented in any other diseases—except cases of manganese poisoning—implying that high brain manganese might be a specific hallmark of prion diseases. This has prompted intensive work on the relationship between prion diseases and environmental pollution. It is proposed that in regions where manganese levels are abnormally high, the manganese may bind to the normal brain PrPC protein and alter its structure into the abnormal PrPSc form. Under these circumstances, the PrPC would lose its protective antioxidant function and predispose the brain to increased oxidative damage.

Many questions concerning the connection between metals and prion diseases remain to be answered. What is clear is that metals can be both beneficial and malicious to the structure and function of PrP. It is important to elucidate the mechanisms involved in these brain metal perturbations and their role in modulating the structure of PrP. Furthermore, it is also essential to determine the structural and functional changes induced by different metals on PrP at the molecular level and the resultant phenotypic features. Conclusive evidence that the loss of PrPC function contributes to prion diseases requires further experiments, possibly with animal models. What is certain is that the next few years will be crucial and exciting in deducing whether brain metal abnormalities constitute a mechanism in the development of prion disease.

See also BSE and CJD: Socio-economic impact and ethical issues; BSE and CJD; Slow virus and viral diseases

BUBONIC PLAGUE

Bubonic plague is a disease that is typically passed from rodents to other animals and humans via the bite of a flea. The flea acquires the bacterium that causes the disease as it lives on the skin of the rodent. Humans can also acquire the disease by direct contact with infected tissue. The bacterium is called *Yersinia pestis*, after one of its co-discoverers, Alexandre Yersin.

The disease is named because of the symptoms. The **bacterial infection** produces a painful swelling of the lymph nodes. These are called buboes. Often the first swelling is evident in the groin. During the Middle Ages, an huge epidemic of bubonic plague was referred to as the Black Death, because of the blackening of the skin due to the dried blood that accumulated under the skin's surface.

The bubonic plague has been a significant cause of misery and death throughout recorded history. The Black Death was only one of many **epidemics** of plague that extend back to the beginning of recorded history. Biblical descriptions of some disease outbreaks likely involved bubonic plague. The first recorded outbreak of bubonic plague was in 542–543. This plague destroyed the attempts of the Roman emperor of the day to re-establish a Roman empire in Europe. This is only one example of how bubonic plague has changed the course of history.

The plague of London in 1665 killed over 17,000 people (almost twenty percent of the city's population). This outbreak was quelled by a huge fire that destroyed most of the city.

The disease remains present to this day. In North America, the last large epidemic occurred in Los Angeles in 1925. With the advent of the antibiotic, era bubonic plague has been controlled in the developed world. However, sporadic cases (e.g., 10 to 15 cases each year) still occur in the western United States. In less developed countries (e.g., in Africa, Bolivia, Peru, Ecuador, Brazil) thousands of cases are reported each year.

The infrequent outbreaks of bubonic plague does not mean the disease disappears altogether. Rather, the disease normally exists in what is called an enzootic state. That is, a few individuals of a certain community (e.g., rodents) harbor the disease. Sometimes, however, environmental conditions cause the disease to spread through the carrier population, causing loss of life. As the rodent populations dies, the fleas that live on them need to find other food sources. This is when the interaction with humans and non-rodent animals can occur. Between outbreaks, *Yersinia pestis* infects rodents without causing much illness. Thus, the rodents become a reservoir of the infection.

Symptoms of infection in humans begin within days after **contamination** with the plague bacterium. The **bacteria** enter the bloodstream and travels to various organs (e.g., kidney, liver, spleen, lungs) as well as to the brain. Symptoms include shivering, nausea with vomiting, headache, intolerance to light, and a whitish-appearing tongue. Buboes then appear, followed by rupture of blood vessels. The released blood can coagulate and turn black.

If the infection is untreated, the death rate in humans approaches 75%. Prompt treatment most often leads to full recovery and a life-long **immunity** from further infection. Prevention is possible, since a **vaccine** is available. Unfortunately, the vaccine is protective for only a few months. Use of the vaccine is usually reserved for those who will be at high risk for acquiring the bacterial infection (e.g., soldiers, travelers to an outbreak region). **Antibiotics** such as tetracycline or sulfonamide are used more commonly as a precaution for those who might be exposed to the bacterium. Such use of antibiotics should be stopped once the risk of infection is gone, to avoid the development of resistance in other bacteria resident in the body.

These modern day treatment and preventative measures are a marked improvement from earlier times. In the fourteenth century, treatments included bathing in human urine, wearing feces, having a dead animal present in the home, and drinking concoctions of molten gold and crushed emeralds. As time progressed, even though the cause of the disease was still unknown, the preventative measures became more constructive. By the fifteenth century, for example, incoming ships were required to anchor offshore for 40 days before cargo or people could disembark. Quarantine is still practiced today as a protective measure for some diseases.

Drawing depicting the effect of Bubonic plague in eighteenth-century London.

The most effective way to prevent bubonic plague is the maintenance of adequate sanitary conditions. This acts to control the rodent population, especially in urban centers.

See also Bacteria and bacterial infection; Epidemics and pandemics; Zoonoses

BUCHANAN, ROBERT EARLE (1883-1972)

American microbial taxonomist

Robert Earle Buchanan's contributions to the wider world of microbiology involve the classification of **microorganisms** and his activity in the expansion of the Society of American Bacteriologists. This expansion was one of the important events that led the Society to become the American Society for Microbiology, the paramount microbiology society in the world.

Buchanan was educated at Iowa State University and subsequently became a faculty member there. He received a B.S. degree in 1904, and a M.S. degree in 1906. After joining the faculty, he became the first head of the Bacteriology Department in 1910. He remained head until his retirement in 1948. He was also the first dean of Industrial Science, first dean of the Graduate College (1919–1948), and was Director of the Agriculture Experiment Station from 1933 until 1948.

In his research life, Buchanan was a microbial taxonomist, concerned with the classification of microorganisms. This interest led him to serve on the Board of Trustees of the *Bergey's Manual of Determinative Bacteriology,* and to assume the responsibilities of co-editor of the eighth edition of the manual in 1974. The Manual is the definitive reference volume on bacterial classification. Buchanan was also one of the founders of the *International Bulletin of Bacterial Nomenclature and Taxonomy* in 1951. He served on the first editorial board of the journal. The journal is still published, now as the *International Journal of Systematic and Evolutionary Bacteriology.*

In 1934, the Society of American Bacteriologists began the process of expansion by adding a branch that represented bacteriologists in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin. Buchanan became the first President of the Northwest Branch of the Society in 1935.

Buchanan also founded the Iowa State *Journal of Science* in 1926. The journal was intended as a forum for the rapid publication of research papers that were too lengthy for publication in other scientific journals. The journal published works from the biological and agricultural sciences and, in 1972, research from the humanities. The journal ceased publication in 1988.

Another landmark publication of Buchanan was in 1960. Then, he published an essay listing the correct Latin forms of chemical elements and compounds that are used in the naming of **bacteria**, yeasts, many filamentous **fungi** and some **protozoa**. This article has proven vital to several generations of bacterial taxonomists.

Buchanan was also active in other international agencies, including the National Research Council, Inter-American Institute of Agricultural Sciences, the President's Committee on Foreign Aid. His service on missions to Greece, the Middle East, and India spread agricultural technology and knowledge of microbiological diseases of agricultural crops around the developing world.

BUDDING • *see* YEAST

BUFFER

A buffer is a solution that resists changes in **pH** upon the addition of acid or base. Buffers typically contain several species that react with added acid and base.

Buffers are important in maintaining the proper environment within **microorganisms** and within other cells, including those in man. In the microbiology laboratory, many solutions and growth media are buffered to prevent sudden and adverse changes in the acidity or alkalinity of the environment surrounding the microorganisms.

Blood is an example of a natural buffer. In water, small volumes of an acid or base solution can greatly change the pH (measure of the hydrogen ion concentration). If the same amount of the acid or base solution is added to blood, the normal pH of the blood (7.4) changes only marginally. Blood and many other bodily fluids are naturally buffered to resist changes in pH.

In order to explain the properties of a buffer, it is useful to consider a specific example, the acetic acid/acetate buffer system. When acid (e.g., HCl, hydrochloric acid) is added to this buffer, the added hydronium ion (H^+) reacts with the strongest base in the medium, namely the acetate ion, to form more acetic acid. This reaction uses up the added hydronium ion, preventing the pH from rising drastically, and is responsible for the buffering effect. As a result of adding acid to the buffer, the concentration of acetate decreases and the concentration of acetic acid increases. The solution acts as a buffer because nearly all of the added hydronium ion is consumed by reaction with acetate. As the hydrogen ion concentration increases, the acetate concentration and acetic acid concentration must adjust. The pH changes slightly to reflect the shift in the concentrations, but the change is much smaller than in the absence of the buffer because most of the added acid is consumed by its reaction with the acetate ion. This example of an acetic acid/acetate ion buffer is typical of other buffer systems.

Buffers are vitally important in living prokaryotic and eukaryotic systems. The rates of various biochemical reactions are very sensitive to the availability of hydronium ions. Many biochemical reactions (e.g., **metabolism**, **respiration**, the transmission of nerve impulses, and muscle contraction and relaxation) take place only within a narrow range of pH.

An important buffer in the blood is the bicarbonate ion and dissolved carbon dioxide in the form of carbonic acid. The acidity or alkalinity of the blood can be altered by the ingestion of acidic or basic substances. The carbonate/bicarbonate

buffer system compensates for such additions and maintains the pH within the required range.

This buffering system is intimately tied to respiration, and an exceptional feature of pH control by this system is the role of ordinary breathing in maintaining the pH. Carbon dioxide is a normal product of metabolism. It is transported to the lungs, where it is eliminated from the body with every exhalation. However, carbon dioxide in blood is converted to carbonic acid, which dissociates to produce the hydrogen carbonate ion and the hydronium ion. If a chemical reaction or the ingestion of an acidic material increases the hydronium ion concentration in the blood, bicarbonate ion reacts with the added hydronium ion and is transformed into carbonic acid. As a result the concentration of dissolved carbon dioxide in the blood increases. Respiration increases, and more carbon dioxide is expelled from the lungs. Conversely, if a base is ingested, the hydronium ion reacts with it, causing a decrease in the concentration of hydronium ion. More carbonic acid dissociates to restore the hydronium ion consumed by the base. This requires more carbon dioxide to be dissolved in the blood, so respiration is decreased and more gas is retained.

To act as a buffer, a solution must maintain a nearly constant pH when either acid or base is added. Two considerations must be made when a buffer is prepared: (1) Which pH is desired to maintain? The desired pH defines the range of the buffer. (2) How much acid or base does the solution need to consume without a significant change in pH? This defines the capacity of the buffer. The desired pH also determines the compounds used in making up the buffer. The quantity of acid or base the buffer must be able to consume determines the concentrations of the components that must be used, and which allows biological reactions to take place consistently.

See also Biochemical analysis techniques; Laboratory techniques in microbiology

Burnet, Frank Macfarlane (1899-1985)

Australian immunologist and virologist

While working at the University of Melbourne's Walter and Eliza Hall Institute for Medical Research in the 1920s, Frank Macfarlane Burnet became interested in the study of **viruses** and **bacteriophage** (viruses that attack **bacteria**). That interest eventually led to two major and related accomplishments. The first of these was the development of a method for cultivating viruses in chicken embryos, an important technological step forward in the science of **virology**. The second accomplishment was the development of a theory that explains how an organism's body is able to distinguish between its own cells and those of another organism. For this research, Burnet was awarded a share of the 1960 Nobel Prize for physiology or medicine (with **Peter Brian Medawar**).

Burnet was born in Traralgon, Victoria, Australia. His father was Frank Burnet, manager of the local bank in Traralgon, and his mother was the former Hadassah Pollock MacKay. As a child, Burnet developed an interest in nature, particularly in birds, butterflies, and beetles. He carried over that interest when he entered Geelong College in Geelong, Victoria, where he majored in biology and medicine.

In 1917, Burnet continued his education at Ormond College of the University of Melbourne, from which he received his bachelor of science degree in 1922 and then, a year later, his M.D. degree. Burnet then took concurrent positions as resident pathologist at the Royal Melbourne Hospital and as researcher at the University of Melbourne's Hall Institute for Medical Research. In 1926, Burnet received a Beit fellowship that permitted him to spend a year in residence at the Lister Institute of Preventive Medicine in London. The work on viruses and bacteriophage that he carried out at Lister also earned him a Ph.D. from the University of London in 1927. At the conclusion of his studies in England in 1928, Burnet returned to Australia, where he became assistant director of the Hall Institute. He maintained his association with the institute for the next thirty-seven years, becoming director there in 1944. In the same year, he was appointed professor of experimental medicine at the University of Melbourne.

Burnet's early research covered a somewhat diverse variety of topics in virology. For example, he worked on the classification of viruses and bacteriophage, on the occurrence of psittacosis in Australian parrots, and on the **epidemiology** of **herpes** and **poliomyelitis**. His first major contribution to virology came, however, during his year as a Rockefeller fellow at London's National Institute for Medical Research from 1932 to 1933. There he developed a method for cultivating viruses in chicken embryos. The Burnet technique was an important breakthrough for virologists since viruses had been notoriously difficult to **culture** and maintain in the laboratory.

Over time, Burnet's work on viruses and bacteriophage led him to a different, but related, field of research, the vertebrate **immune system**. The fundamental question he attacked is one that had troubled biologists for years: how an organism's body can tell the difference between "self" and "not-self." An organism's immune system is a crucial part of its internal hardware. It provides a mechanism for fighting off invasions by potentially harmful—and sometimes fatal—foreign organisms (antigens) such as bacteria, viruses, and **fungi**. The immune system is so efficient that it even recognizes and fights back against harmless invaders such as pollen and dust, resulting in allergic reactions.

Burnet was attracted to two aspects of the phenomenon of **immunity**. First, he wondered how an organism's body distinguishes between foreign invaders and components of its own body, the "self" versus "not-self" problem. That distinction is obviously critical, since if the body fails to recognize that difference, it may begin to attack its own cells and actually destroy itself. This phenomenon does, in fact, occur in some cases of **autoimmune disorders**.

The second question on which Burnet worked was how the immune system develops. The question is complicated by the fact that a healthy immune system is normally able to recognize and respond to an apparently endless variety of antigens, producing a specific chemical (**antibody**) to combat each **antigen** it encounters. According to one theory, these antibod-

ies are present in an organism's body from birth, prior to birth, or an early age. A second theory suggested that antibodies are produced "on the spot" as they are needed and in response to an attack by an antigen.

For more than two decades, Burnet worked on resolving these questions about the immune system. He eventually developed a complete and coherent explanation of the way the system develops in the embryo and beyond, how it develops the ability to recognize its own cells as distinct from foreign cells, and how it carries with it from the very earliest stages the templates from which antibodies are produced. For this work, Burnet was awarded a share of the 1960 Nobel Prize in physiology or medicine. Among the other honors he received were the Royal Medal and the Copley Medal of the Royal Society (1947 and 1959, respectively) and the Order of Merit in 1958. He was elected a fellow of the Royal Society in 1947 and knighted by King George V in 1951.

Burnet retired from the Hall Institute in 1965, but continued his research activities. His late work was in the area of autoimmune disorders, cancer, and aging. He died of cancer in Melbourne in 1985. Burnet was a prolific writer, primarily of books on science and medicine, during his lifetime.

See also Antigens and antibodies; Autoimmune disorders; Bacteriophage and bacteriophage typing; Immunity, cell mediated; Immunity, humoral regulation; Virology; Virus replication; Viruses and responses to viral infection

C

CAMPYLOBACTERIOSIS

Campylobacteriosis is a **bacterial infection** of the intestinal tract of humans. The infection, which typically results in diarrhea, is caused by members of the genus *Campylobacter.* In particular, *Campylobacter jejuni* is the most commonly cause of bacterial diarrhea in the United States (and likely other countries as well), with more occurrences than **salmonella** (another prominent disease causing **bacteria** associated with food poisoning). Worldwide, approximately five to fourteen per cent of all diarrhea is thought to be the result of campylobacteriosis.

Humans contract campylobacteriosis by eating or drinking contaminated food or water. Less often, direct contact with infected people or animals can spread the infection. The infection begins from two to five days after the contaminated food or water has been ingested.

The illness caused by *Campylobacter* bacteria has been known for decades, and was recognized as a cause of disease in animals since 1909. However, it is only in the last two decades of the twentieth century that the bacteria were identified as the cause of the human disease campylobacteriosis. Over 10,000 cases are now reported to the Unites States **Centers for Disease Control** (CDC) each year. As the illness is often not identified, the actual number of cases is much higher. Indeed, CDC estimates that 2 million people contract campylobacteriosis each year in the United States.

In under-developed countries, campylobacteriosis is a significant health threat. Organization such as the **World Health Organization** have devoted much effort to improving the **water quality** of villages in an effort to decrease the incidence of water-borne campylobacteriosis.

The *Campylobacter* organism is distinctive on several counts. The bacteria have a spiral shape. Also, they are fragile, not tolerating drying or the presence of pure oxygen.

As with other bacterial intestinal upsets, campylobacteriosis is more of a transient inconvenience than a dire health threat in the developed world. The symptoms of the disease (malaise, fever, abdominal cramps, diarrhea, nausea, vomiting) are often mistaken for stomach flu. Still, severe forms of the infection can produce bloody diarrhea. In some people, especially in infants, the elderly, and those whose immune systems are not operating efficiently, the resulting diarrhea and fluid loss can produce dehydration if fluid intake is not maintained during the period of illness. Very rarely, seizures can occur due to high fever or because of the exacerbation of a neurological disorder such as Guillain-Barre syndrome. Guillan-Barre syndrome occurs when a person's own **immune system** begins to attack the body's own nerves. Paralysis can result. It has been estimated that one in every 1000 cases of campylobacteriosis leads to Guillan-Barre syndrome.

Most people afflicted with campylobacteriosis recover on their own. Occasionally, **antibiotics** need to be given to rid the body of the infection. While the main bout of the malady passes in about a week, abdominal cramps can recur for up to three months after an infection.

Campylobacteriosis is an example of a zoonosis (an ailment passed to humans via animals or animal products). *Campylobacter* bacteria naturally inhabit the intestinal tract of many animals, including swine, cattle, ostriches, dogs, shellfish and poultry. These creatures can carry the bacteria without displaying any symptoms of illness. Soil is another habitat. A principle reason for the wide distribution of *Campylobacter* is the ability of the bacteria to survive anywhere there is moisture, food source, less than an atmospheric level of oxygen and room temperature conditions. In particular, poultry are a reservoir of the microorganism. These sources can contaminate meat products, water and milk. Studies monitoring poultry carcasses in processing plants have demonstrated that over 50% of raw chicken is contaminated with *Campylobacter.*

The prevalence of *Campylobacter jejuni* in poultry carcasses results from the **contamination** of the meat by the intestinal contents of the bird (including the bacteria) when an infected bird is slaughtered. Because chickens can carry the organism without showing any symptoms of infection, they can escape inspection.

Despite the high contamination rate of foodstuffs such as poultry, *Campylobacter jejuni* does not grow readily on or in foods. Furthermore, the organism is sensitive to temperatures much above room temperature. Proper cooking of food will readily destroy the bacteria. Other sensible hygienic practices, such as washing the cutting board after dealing with a chicken, also reduce the chances of illness. Unfortunately, undercooking of foods such as poultry, poor **hygiene**, and inadequate **disinfection** of drinking water accounts for most of the cases of campylobacteriosis.

See also Food safety; Water quality

CANDIDIASIS

Candidiasis is an infection that is caused by members of the fungal genus *Candida*.

The two most common species associated with Candidiasis are *Candida albicans* and *Candida glabrata*. Less commonly, but still able to cause the infection, are *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondi*, and *Candida krusei*.

The fungus is a normal resident of the body, typically in the mouth and the gastrointestinal tract. In these habitats, the microorganism normally colonizes the cell surface. In healthy people in the United States, *Candida* species colonize more than half of these individuals. The presence of the fungus is beneficial. Invading **bacteria** are recognized by the *Candida* cells and are destroyed. Thus, the **fungi complement** the **immune system** and other defenses of the body against infection.

When the body is in proper balance with respect to the microbial flora, the fungi exist as a so-called **yeast** form. These are not capable of invasion. However, *Candida* can infect areas of the body that are warm and moist. These include the eye (conjunctivitis), fingernails, rectum, folds in the skin, and, in infants, the skin irritation in infants known commonly as diaper rash. Typically, such infections are more of an inconvenience than a dangerous health concern.

However, in people whose immune systems are compromised in some way, or when the normal balance of the microbial flora has been disrupted by, for example, antibiotic therapy, *Candida* can establish an infection. For example, an infection of the mouth region, which is referred to as oropharyngeal infection, was a very common infection in those whose immune system was deficient due to infection with the **Human immunodeficiency virus**. More aggressive antiviral therapy has reduced the incidence of the infection.

Such infections are associated with the change from the *Candida* cells from the yeast form to a so-called mycelial fungal form. The mycelia produce long, root-like structures that are called rhizoids. The rhizoids can penetrate through the mucous cells that line the inside of the mouth and vagina, and through the epithelial cells that line the intestinal tract. This invasion can spread the infection to the bloodstream. As well, the microscopic holes that are left behind in the cell walls can be portals for the entry of toxins, undigested food, bacteria, and yeast.

In countries around the world where fungal infections are widespread in the populations, *Candida* species have overtaken *Cryptococcus* species as the most common cause of infections that affect the central nervous system of immunocompromised people.

Besides the oropharyngeal infection, *Candida* can also commonly cause a vaginal infection. Both infections are evident by the development of a fever and chills that, because of the fungal genesis of the infections, are unaffected by antibacterial therapy. Visually, white patches appear on the surface of the cells lining the mouth and oral cavity and the vagina. More rarely, the infections may spread to the bloodstream. Examples of the infections that can result include the kidney, spleen, nerve cells (**meningitis**), heart (endocarditis). Arthritis may even develop. Immunocompromised individuals are especially susceptible to these infections.

The **contamination** of the bloodstream by *Candida* occurs most commonly in the hospital setting, where a patient is being treated for Candidiasis or other malady. Indeed, this type of bloodstream infection is the fourth most common cause of hospital-acquired bloodstream infections in the United States. The death rate from the infection can approach 40 per cent.

Treatment for *Candida* infections consist of the administration of antifungal drugs. Examples of the drugs of choice include amphotericin B, fluconazole, ketoconazole, and nystatin. The real possibility of the development of irritative side effects makes monitoring during therapy a prudent precaution.

See also Fungi; Immunodeficiency

CAPSID AND CAPSULE • *see* GLYCOCALYX

CARBON CYCLE IN MICROORGANISMS

The carbon cycle in **microorganisms** is part of a larger cycling of carbon that occurs on the global scale. The actions of microorganisms help extract carbon from non-living sources and make the carbon available to living organisms (including themselves).

The cycling of carbon by microorganisms, including a variety of **bacteria** and **fungi**, occurs in aquatic habitats. Even relatively oxygen-free zones such as in the deep mud of lakes, ponds and other water bodies can be regions where the anaerobic conversion of carbon takes place.

Much of the carbon that enters the carbon cycle of microorganisms is carbon dioxide. This form of carbon exists as a gas in the atmosphere and can be dissolved in water. The atmospheric carbon dioxide can be converted to organic material in the process of **photosynthesis**. Photosynthetic algae are important microorganisms in this regard. As well, chemoautotrophs, primarily bacteria and archae are capable of carbon dioxide conversion. In both systems the carbon dioxide is converted to chains that are comprised of sugars that have the structure CH_2O.

Both types of conversion take place in the presence and the absence of oxygen. Algal involvement is an aerobic process. The conversion of carbon dioxide to sugar is an energy-requiring process that generates oxygen as a by-product. This **evolution** of oxygen also occurs in plants and is one of the recognized vital benefits of trees to life on Earth.

The carbon available in the carbohydrate sugar molecules is cycled further by microorganisms in a series of reactions that form the so-called tricarboxylic acid (or TCA) cycle. The breakdown of the carbohydrate serves to supply energy to the microorganism. This process is also known as **respiration**. In anaerobic environments, microorganisms can cycle the carbon compounds to yield energy in a process known as **fermentation**.

Carbon dioxide can be converted to another gas called methane (CH_4). This occurs in anaerobic environments, such as deep compacted mud, and is accomplished by bacteria known as methanogenic bacteria. The conversion, which requires hydrogen, yields water and energy for the methanogens. To complete the recycling pattern another group of methane bacteria called methane-oxidizing bacteria or methanotrophs (literally "methane eaters") can convert methane to carbon dioxide. This conversion, which is an aerobic (oxygen-requiring) process, also yields water and energy. Methanotrophs tend to live at the boundary between aerobic and anaerobic zones. There they have access to the methane produced by the anaerobic methanogenic bacteria, but also access to the oxygen needed for their conversion of the methane.

Other microorganisms are able to participate in the cycling of carbon. For example the green and purple sulfur bacteria are able to use the energy they gain from the degradation of a compound called hydrogen sulfide to degrade carbon compounds. Other bacteria such as *Thiobacillus ferrooxidans* uses the energy gained from the removal of an electron from iron-containing compounds to convert carbon.

The anerobic degradation of carbon is done only by microorganisms. This degradation is a collaborative effort involving numerous bacteria. Examples of the bacteria include *Bacteroides succinogenes*, *Clostridium butyricum*, and *Syntrophomonas sp*. This bacterial collaboration, which is termed interspecies hydrogen transfer, is responsible for the bulk of the carbon dioxide and methane that is released to the atmosphere.

See also Bacterial growth and division; Chemoautotrophic and chemolithotrophic bacteria; Metabolism; Methane oxidizing and producing bacteria; Nitrogen cycle in microorganisms

CAULOBACTER

Caulobacter crescentus is a Gram-negative rod-like bacterium that inhabits fresh water. It is noteworthy principally because of the unusual nature of its division. Instead of dividing two form two identical daughter cells as other **bacteria** do (a process termed binary division), *Caulobacter crescentus* undergoes what is termed symmetric division. The parent bacterium divides to yield two daughter cells that differ from one another structurally and functionally.

When a bacterium divides, one cell is motile by virtue of a single flagellum at one end. This daughter cell is called a swarmer cell. The other cell does not have a flagellum. Instead, at one end of the cell there is a stalk that terminates in an attachment structure called a holdfast. This daughter cell is called the stalk cell. The stalk is an outgrowth of the cell wall, and serves to attach the bacterium to plants or to other microbes in its natural environment (lakes, streams, and sea water).

Caulobacter crescentus exhibits a distinctive behavior. The swarmer cell remains motile for 30 to 45 minutes. The cell swims around and settles onto a new surface where the food supply is suitable. After settling, the flagellum is shed and the bacterium differentiates into a stalk cell. With each division cycle the stalk becomes longer and can grow to be several times as long as the body of the bacterium.

The regulation of **gene** expression is different in the swarmer and stalk cells. Replication of the genetic material occurs immediately in the stalk cell but for reasons yet to be determined is repressed in the swarmer cell. However, when a swarmer cell differentiates into a stalk cell, replication of the genetic material immediately commences. Thus, the transition to a stalk cell is necessary before division into the daughter swarmer and stalk cells can occur.

The genetics of the swarmer to stalk **cell cycle** are complex, with at least 500 genes known to play a role in the structural transition. The regulation of these activities with respect to time are of great interest to geneticists.

Caulobacter crescentus can be grown in the laboratory so that all the bacteria in the population undergoes division at the same time. This type of growth is termed **synchronous growth**. This has made the bacterium an ideal system to study the various events in gene regulation necessary for growth and division.

See also Bacterial appendages; Bacterial surface layers; Cell cycle (prokaryotic), genetic regulation of; Phenotypic variation

CDC

• *see* CENTERS FOR DISEASE CONTROL (CDC)

CECH, THOMAS R. (1947-)

American biochemist

The work of Thomas R. Cech has revolutionized the way in which scientists look at **RNA** and at proteins. Up to the time of Cech's discoveries in 1981 and 1982, it had been thought that genetic coding, stored in the **DNA** of the **nucleus**, was imprinted or transcribed onto RNA molecules. These RNA molecules, it was believed, helped transfer the coding onto proteins produced in the **ribosomes**. The DNA/RNA nexus was thus the information center of the cell, while protein molecules in the form of **enzymes** were the workhorses, catalyzing the thousands of vital chemical reactions that occur in the cell. Conventional wisdom held that the two functions were

separate—that there was a delicate division of labor. Cech and his colleagues at the University of Colorado established, however, that this picture of how RNA functions was incorrect; they proved that in the absence of other enzymes RNA acts as its own catalyst. It was a discovery that reverberated throughout the scientific community, leading not only to new technologies in RNA engineering but also to a revised view of the **evolution** of life. Cech shared the 1989 Nobel Prize for Chemistry with Sidney Altman at Yale University for their work regarding the role of RNA in cell reactions.

Cech was born in Chicago, Illinois, to Robert Franklin Cech, a physician, and Annette Marie Cerveny Cech. Cech recalled in an autobiographical sketch for *Les Prix Nobel,* he grew up in "the safe streets and good schools" of Iowa City, Iowa. His father had a deep and abiding interest in physics as well as medicine, and from an early age Cech took an avid interest in science, collecting rocks and minerals and speculating about how they had been formed. In junior high school he was already conferring with geology professors from the nearby university. Cech went to Grinnell College in 1966; at first attracted to physical chemistry, he soon concentrated on biological chemistry, graduating with a chemistry degree in 1970.

It was at Grinnell that he met Carol Lynn Martinson, who was a fellow chemistry student. They married in 1970 and went together to the University of California at Berkeley for graduate studies. His thesis advisor there was John Hearst who, Cech recalled in *Les Prix Nobel,* "had an enthusiasm for chromosome structure and function that proved infectious." Both Cech and his wife were awarded their Ph.D. degrees in 1975, and they moved to the east coast for postdoctoral positions—Cech at the Massachusetts Institute of Technology (MIT) under Mary Lou Pardue, and his wife at Harvard. At MIT Cech focused on the DNA structures of the mouse genome, strengthening his knowledge of biology at the same time.

In 1978, both Cech and his wife were offered positions at the University of Colorado in Boulder; he was appointed assistant professor in chemistry. By this time, Cech had decided that he would like to investigate more specific genetic material. He was particularly interested in what enables the DNA molecule to instruct the body to produce the various parts of itself—a process known as **gene** expression. Cech set out to discover the proteins that govern the DNA **transcription** process onto RNA, and in order to do this he decided to use nucleic acids from a single-cell **protozoa**, *Tetrahymena thermophila*. Cech chose *Tetrahymena* because it rapidly reproduced genetic material and because it had a structure which allowed for the easy extraction of DNA.

By the late 1970s, much research had already been done on DNA and its transcription partner, RNA. It had been determined that there were three types of RNA: messenger RNA, which relays the transcription of the DNA structure by attaching itself to the ribosome where **protein synthesis** occurs; ribosomal RNA, which imparts the messenger's structure within the ribosome; and transfer RNA, which helps to establish amino acids in the proper order in the protein chain as it is being built. Just prior to the time Cech began his work, it was discovered that DNA and final-product RNA (after copying or transcription) actually differed. In 1977, Phillip A. Sharp and others discovered that portions of seemingly noncoded DNA were snipped out of the RNA and the chain was spliced back together where these intervening segments had been removed. These noncoded sections of DNA were called introns.

Cech and his coworkers were not initially interested in such introns, but they soon became fascinated with their function and the splicing mechanism itself. In an effort to understand how these so-called nonsense sequences, or introns, were removed from the transcribed RNA, Cech and his colleague Arthur Zaug decided to investigate the pre-ribosomal RNA of the *Tetrahymena,* just as it underwent transcription. In order to do this, they first isolated unspliced RNA and then added some *Tetrahymena* nuclei extract. Their assumption was that the catalytic agent or enzyme would be present in such an extract. The two scientists also added small molecules of salts and nucleotides for energy, varying the amounts of each in subsequent experiments, even excluding one or more of the additives. But the experiment took a different turn than was expected.

Cech and Zaug discovered instead that RNA splicing occurred even without the nucleic material being present. This was a development they did not understand at first; it was a long-held scientific belief that proteins in the form of enzymes had to be present for catalysis to occur. Presenting itself was a situation in which RNA appeared to be its own catalytic motivator. At first they suspected that their experiment had been contaminated. Cech did further experiments involving recombinant DNA in which there could be no possibility of the presence of splicing enzymes, and these had the same result: the RNA spliced out its own intron. Further discoveries in Cech's laboratory into the nature of the intron led to his belief that the intron itself was the catalytic agent of RNA splicing, and he decided that this was a sort of RNA enzyme which they called the ribozyme.

Cech's findings of 1982 met with heated debate in the scientific community, for it upset many beliefs about the nature of enzymes. Cech's ribozyme was in fact not a true enzyme, for thus far he had shown it only to work upon itself and to be changed in the reaction; true enzymes catalyze repeatedly and come out of the reaction unchanged. Other critics argued that this was a freak bit of RNA on a strange microorganism and that it would not be found in other organisms. The critics were soon proved wrong, however, when scientists around the world began discovering other RNA enzymes. In 1984, Sidney Altman proved that RNA carries out enzyme-like activities on substances other than itself.

The discovery of catalytic RNA has had profound results. In the medical field alone RNA enzymology may lead to cures of viral infections. By using these rybozymes as gene scissors, the RNA molecule can be cut at certain points, destroying the RNA molecules that cause infections or genetic disorders. In life sciences, the discovery of catalytic RNA has also changed conventional wisdom. The old debate about whether proteins or nucleic acids were the first bit of life form seems to have been solved. If RNA can act as a catalyst and a genetic template to create proteins as well as itself, then it is rather certain that RNA was first in the chain of life.

Cech and Altman won the Nobel Prize for chemistry in 1989 for their independent discoveries of catalytic RNA. Cech

has also been awarded the Passano Foundation Young Scientist Award and the Harrison Howe Award in 1984; the Pfizer Award in Enzyme Chemistry in 1985; the U. S. Steel Award in **Molecular Biology**; and the V. D. Mattia Award in 1987. In 1988, he won the Newcombe-Cleveland Award, the Heineken Prize, the Gairdner Foundation International Award, the Louisa Gross Horwitz Prize, and the Albert Lasker Basic Medical Research Award; he was presented with the Bonfils-Stanton Award for Science in 1990.

Cech was made full professor in the department of chemistry at the University of Colorado in 1983. Cech and his wife have two daughters. In the midst of his busy research career, Cech finds time to enjoy skiing and backpacking.

See also Viral genetics

CELL-MEDIATED IMMUNE RESPONSE • *see* IMMUNITY, CELL MEDIATED

CELL CYCLE AND CELL DIVISION

The series of stages that a cell undergoes while progressing to division is known as cell cycle. In order for an organism to grow and develop, the organism's cells must be able to duplicate themselves. Three basic events must take place to achieve this duplication: the **deoxyribonucleic acid DNA**, which makes up the individual **chromosomes** within the cell's **nucleus** must be duplicated; the two sets of DNA must be packaged up into two separate nuclei; and the cell's **cytoplasm** must divide itself to create two separate cells, each complete with its own nucleus. The two new cells, products of the single original cell, are known as daughter cells.

Although prokaryotes (e.g. **bacteria**, non-nucleated unicellular organisms) divide through binary fission, **eukaryotes** (including, of course, human cells) undergo a more complex process of cell division because DNA is packed in several chromosomes located inside a cell nucleus. In eukaryotes, cell division may take two different paths, in accordance with the cell type involved. Mitosis is a cellular division resulting in two identical nuclei that takes place in somatic cells. Sex cells or gametes (ovum and spermatozoids) divide by meiosis. The process of meiosis results in four nuclei, each containing half of the original number of chromosomes. Both prokaryotes and eukaryotes undergo a final process, known as cytoplasmatic division, which divides the parental cell in new daughter cells.

Mitosis is the process during which two complete, identical sets of chromosomes are produced from one original set. This allows a cell to divide during another process called cytokinesis, thus creating two completely identical daughter cells.

During much of a cell's life, the DNA within the nucleus is not actually organized into the discrete units known as chromosomes. Instead, the DNA exists loosely within the nucleus, in a form called chromatin. Prior to the major events of mitosis, the DNA must replicate itself, so that each cell has twice as much DNA as previously.

Cells undergoing division are also termed competent cells. When a cell is not progressing to mitosis, it remains in phase G0 ("G" zero). Therefore, the cell cycle is divided into two major phases: interphase and mitosis. Interphase includes the phases (or stages) G1, S and G2 whereas mitosis is subdivided into prophase, metaphase, anaphase and telophase.

Interphase is a phase of cell growth and metabolic activity, without cell nuclear division, comprised of several stages or phases. During Gap 1 or G1 the cell resumes protein and **RNA** synthesis, which was interrupted during previous mitosis, thus allowing the growth and maturation of young cells to accomplish their physiologic function. Immediately following is a variable length pause for DNA checking and repair before cell cycle transition to phase S during which there is synthesis or semi-conservative replication or synthesis of DNA. During Gap 2 or G2, there is increased RNA and **protein synthesis**, followed by a second pause for proofreading and eventual repairs in the newly synthesized DNA sequences before transition to mitosis.

The cell cycle starts in G1, with the active synthesis of RNA and proteins, which are necessary for young cells to grow and mature. The time G1 lasts, varies greatly among eukaryotic cells of different species and from one tissue to another in the same organism. Tissues that require fast cellular renovation, such as mucosa and endometrial epithelia, have shorter G1 periods than those tissues that do not require frequent renovation or repair, such as muscles or connective tissues.

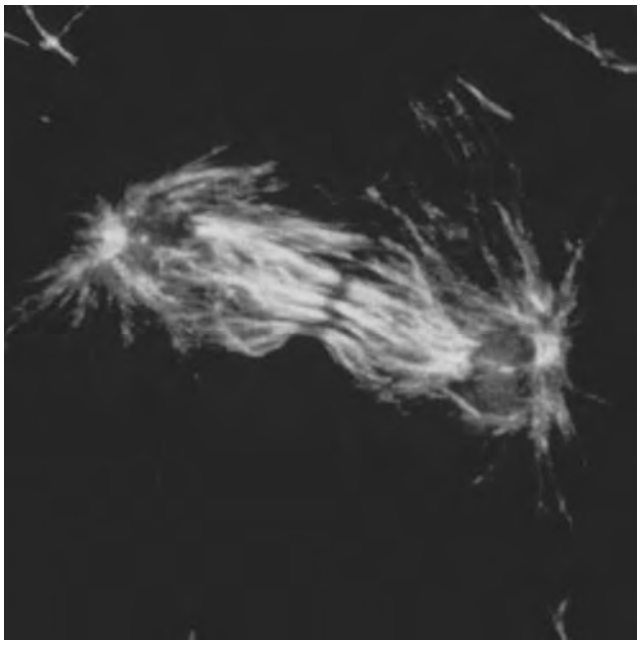
The first stage of mitosis is called prophase. During prophase, the DNA organizes or condenses itself into the specific units known as chromosomes. Chromosomes appear as double-stranded structures. Each strand is a replica of the other and is called a chromatid. The two chromatids of a chromosome are joined at a special region, the centromere. Structures called centrioles position themselves across from each other, at either end of the cell. The nuclear membrane then disappears.

During the stage of mitosis called metaphase, the chromosomes line themselves up along the midline of the cell. Fibers called spindles attach themselves to the centromere of each chromosome.

During the third stage of mitosis, called anaphase, spindle fibers will pull the chromosomes apart at their centromere (chromosomes have two complementary halves, similar to the two nonidentical but complementary halves of a zipper). One arm of each chromosome will migrate toward each centriole, pulled by the spindle fibers.

During the final stage of mitosis, telophase, the chromosomes decondense, becoming unorganized chromatin again. A nuclear membrane forms around each daughter set of chromosomes, and the spindle fibers disappear. Sometime during telophase, the cytoplasm and cytoplasmic membrane of the cell split into two (cytokinesis), each containing one set of chromosomes residing within its nucleus.

Cells are mainly induced into proliferation by growth factors or hormones that occupy specific receptors on the surface of the cell membrane, being also known as extra-cellular



Segregation of eukaryotic genetic material during mitosis.

ligands. Examples of growth factors are as such: epidermal growth factor (EGF), fibroblastic growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), or by hormones. PDGF and FGF act by regulating the phase G2 of the cell cycle and during mitosis. After mitosis, they act again stimulating the daughter cells to grow, thus leading them from G0 to G1. Therefore, FGF and PDGF are also termed competence factors, whereas EGF and IGF are termed progression factors, because they keep the process of cellular progression to mitosis going on. Growth factors are also classified (along with other molecules that promote the cell cycle) as pro-mitotic signals. Hormones are also pro-mitotic signals. For example, thyrotrophic hormone, one of the hormones produced by the pituitary gland, induces the proliferation of thyroid gland's cells. Another pituitary hormone, known as growth hormone or somatotrophic hormone (STH), is responsible by body growth during childhood and early adolescence, inducing the lengthening of the long bones and protein synthesis. Estrogens are hormones that do not occupy a membrane receptor, but instead, penetrate the cell and the nucleus, binding directly to specific sites in the DNA, thus inducing the cell cycle.

Anti-mitotic signals may have several different origins, such as cell-to-cell adhesion, factors of adhesion to the extracellular matrix, or soluble factor such as TGF beta (tumor growth factor beta), which inhibits abnormal cell proliferation, proteins p53, p16, p21, APC, pRb, etc. These molecules are the products of a class of genes called tumor suppressor genes. Oncogenes, until recently also known as proto-oncogenes, synthesize proteins that enhance the stimuli started by growth factors, amplifying the mitotic signal to the nucleus, and/or promoting the accomplishment of a necessary step of the cell cycle. When each phase of the cell cycle is completed, the proteins involved in that phase are degraded, so that once the next phase starts, the cell is unable to go back to the previous one. Next to the end of phase G1, the cycle is paused by tumor suppressor **gene** products, to allow verification and repair of DNA damage. When DNA damage is not repairable, these genes stimulate other intra-cellular pathways that induce the cell into suicide or apoptosis (also known as programmed cell death). To the end of phase G2, before the transition to mitosis, the cycle is paused again for a new verification and "decision": either mitosis or apoptosis.

Along each pro-mitotic and anti-mitotic intra-cellular signaling pathway, as well as along the apoptotic pathways, several gene products (**proteins and enzymes**) are involved in an orderly sequence of activation and inactivation, forming complex webs of signal transmission and signal amplification to the nucleus. The general goal of such cascades of signals is to achieve the orderly progression of each phase of the cell cycle.

Mitosis always creates two completely identical cells from the original cell. In mitosis, the total amount of DNA doubles briefly, so that the subsequent daughter cells will ultimately have the exact amount of DNA initially present in the original cell. Mitosis is the process by which all of the cells of the body divide and therefore reproduce. The only cells of the body that do not duplicate through mitosis are the sex cells (egg and sperm cells). These cells undergo a slightly different type of cell division called meiosis, which allows each sex cell produced to contain half of its original amount of DNA, in anticipation of doubling it again when an egg and a sperm unite during the course of conception.

Meiosis, also known as reduction division, consists of two successive cell divisions in diploid cells. The two cell divisions are similar to mitosis, but differ in that the chromosomes are duplicated only once, not twice. The result of meiosis is four haploid daughter cells. Because meiosis only occurs in the sex organs (gonads), the daughter cells are the gametes (spermatozoa or ova), which contain hereditary material. By halving the number of chromosomes in the sex cells, meiosis assures that the fusion of maternal and paternal gametes at fertilization will result in offspring with the same chromosome number as the parents. In other words, meiosis compensates for chromosomes doubling at fertilization. The two successive nuclear divisions are termed as meiosis I and meiosis II. Each is further divided into four phases (prophase, metaphase, anaphase, and telophase) with an intermediate phase (interphase) preceding each nuclear division.

The events that take place during meiosis are similar in many ways to the process of mitosis, in which one cell divides to form two clones (exact copies) of itself. It is important to note that the purpose and final products of mitosis and meiosis are very different.

Meiosis I is preceded by an interphase period in which the DNA replicates (makes an exact duplicate of itself), resulting in two exact copies of each chromosome that are firmly attached at one point, the centromere. Each copy is a sister chromatid, and the pair are still considered as only one chromosome. The first phase of meiosis I, prophase I, begins as the chromosomes come together in homologous pairs in a process known as synapsis. Homologous chromosomes, or homo-

logues, consist of two chromosomes that carry genetic information for the same traits, although that information may hold different messages (e.g., when two chromosomes carry a message for eye color, but one codes for blue eyes while the other codes for brown). The fertilized eggs (zygotes) of all sexually reproducing organisms receive their chromosomes in pairs, one from the mother and one from the father. During synapsis, adjacent chromatids from homologous chromosomes "cross over" one another at random points and join at spots called chiasmata. These connections hold the pair together as a tetrad (a set of four chromatids, two from each homologue). At the chiasmata, the connected chromatids randomly exchange bits of genetic information so that each contains a mixture of maternal and paternal genes. This "shuffling" of the DNA produces a tetrad, in which each of the chromatids is different from the others, and a gamete that is different from others produced by the same parent. Crossing over does explain why each person is a unique individual, different even from those in the immediate family. Prophase I is also marked by the appearance of spindle fibers (strands of microtubules) extending from the poles or ends of the cell as the nuclear membrane disappears. These spindle fibers attach to the chromosomes during metaphase I as the tetrads line up along the middle or equator of the cell. A spindle fiber from one pole attaches to one chromosome while a fiber from the opposite pole attaches to its homologue. Anaphase I is characterized by the separation of the homologues, as chromosomes are drawn to the opposite poles. The sister chromatids are still intact, but the homologous chromosomes are pulled apart at the chiasmata. Telophase I begins as the chromosomes reach the poles and a nuclear membrane forms around each set. Cytokinesis occurs as the cytoplasm and organelles are divided in half and the one parent cell is split into two new daughter cells. Each daughter cell is now haploid (n), meaning it has half the number of chromosomes of the original parent cell (which is diploid-2n). These chromosomes in the daughter cells still exist as sister chromatids, but there is only one chromosome from each original homologous pair.

The phases of meiosis II are similar to those of meiosis I, but there are some important differences. The time between the two nuclear divisions (interphase II) lacks replication of DNA (as in interphase I). As the two daughter cells produced in meiosis I enter meiosis II, their chromosomes are in the form of sister chromatids. No crossing over occurs in prophase II because there are no homologues to synapse. During metaphase II, the spindle fibers from the opposite poles attach to the sister chromatids (instead of the homologues as before). The chromatids are then pulled apart during anaphase II. As the centromeres separate, the two single chromosomes are drawn to the opposite poles. The end result of meiosis II is that by the end of telophase II, there are four haploid daughter cells (in the sperm or ova) with each chromosome now represented by a single copy. The distribution of chromatids during meiosis is a matter of chance, which results in the concept of the law of independent assortment in genetics.

The events of meiosis are controlled by a protein enzyme complex known collectively as maturation promoting factor (MPF). These **enzymes** interact with one another and with cell organelles to cause the breakdown and reconstruction of the nuclear membrane, the formation of the spindle fibers, and the final division of the cell itself. MPF appears to work in a cycle, with the proteins slowly accumulating during interphase, and then rapidly degrading during the later stages of meiosis. In effect, the rate of synthesis of these proteins controls the frequency and rate of meiosis in all sexually reproducing organisms from the simplest to the most complex.

Meiosis occurs in humans, giving rise to the haploid gametes, the sperm and egg cells. In males, the process of gamete production is known as spermatogenesis, where each dividing cell in the testes produces four functional sperm cells, all approximately the same size. Each is propelled by a primitive but highly efficient flagellum (tail). In contrast, in females, oogenesis produces only one surviving egg cell from each original parent cell. During cytokinesis, the cytoplasm and organelles are concentrated into only one of the four daughter cells—the one that will eventually become the female ovum or egg. The other three smaller cells, called polar bodies, die and are reabsorbed shortly after formation. The concentration of cytoplasm and organelles into the oocyte greatly enhances the ability of the zygote (produced at fertilization from the unification of the mature ovum with a spermatozoa) to undergo rapid cell division.

The control of cell division is a complex process and is a topic of much scientific research. Cell division is stimulated by certain kinds of chemical compounds. Molecules called **cytokines** are secreted by some cells to stimulate others to begin cell division. Contact with adjacent cells can also control cell division. The phenomenon of contact inhibition is a process where the physical contact between neighboring cells prevents cell division from occurring. When contact is interrupted, however, cell division is stimulated to close the gap between cells. Cell division is a major mechanism by which organisms grow, tissues and organs maintain themselves, and wound healing occurs.

Cancer is a form of uncontrolled cell division. The cell cycle is highly regulated by several enzymes, proteins, and cytokines in each of its phases, in order to ensure that the resulting daughter cells receive the appropriate amount of genetic information originally present in the parental cell. In the case of somatic cells, each of the two daughter cells must contain an exact copy of the original genome present in the parental cell. Cell cycle controls also regulate when and to what extent the cells of a given tissue must proliferate, in order to avoid abnormal cell proliferation that could lead to dysplasia or tumor development. Therefore, when one or more of such controls are lost or inhibited, abnormal overgrowth will occur and may lead to impairment of function and disease.

See also Amino acid chemistry; Bacterial growth and division; Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); Enzymes; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Molecular biology and molecular genetics

CELL CYCLE (EUKARYOTIC), GENETIC REGULATION OF

Although prokaryotes (i.e., non-nucleated unicellular organisms) divide through binary fission, **eukaryotes** undergo a more complex process of cell division because **DNA** is packed in several **chromosomes** located inside a cell **nucleus**. In eukaryotes, cell division may take two different paths, in accordance with the cell type involved. Mitosis is a cellular division resulting in two identical nuclei is performed by somatic cells. The process of meiosis results in four nuclei, each containing half of the original number of chromosomes. Sex cells or gametes (ovum and spermatozoids) divide by meiosis. Both prokaryotes and eukaryotes undergo a final process, known as cytoplasmatic division, which divides the parental cell into new daughter cells.

The series of stages that a cell undergoes while progressing to division is known as **cell cycle**. Cells undergoing division are also termed competent cells. When a cell is not progressing to mitosis, it remains in phase G0 ("G" zero). Therefore, the cell cycle is divided into two major phases: interphase and mitosis. Interphase includes the phases (or stages) G1, S and G2 whereas mitosis is subdivided into prophase, metaphase, anaphase and telophase.

The cell cycle starts in G1, with the active synthesis of **RNA** and proteins, which are necessary for young cells to grow and mature. The time G1 lasts, varies greatly among eukaryotic cells of different species and from one tissue to another in the same organism. Tissues that require fast cellular renovation, such as mucosa and endometrial epithelia, have shorter G1 periods than those tissues that do not require frequent renovation or repair, such as muscles or connective tissues.

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Cells are mainly induced into proliferation by growth factors or hormones that occupy specific receptors on the surface of the cell membrane, and are also known as extra-cellular ligands. Examples of growth factors are as such: epidermal growth factor (EGF), fibroblastic growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), or by hormones. PDGF and FGF act by regulating the phase G2 of the cell cycle and during mitosis. After mitosis, they act again stimulating the daughter cells to grow, thus leading them from G0 to G1. Therefore, FGF and PDGF are also termed competence factors, whereas EGF and IGF are termed progression factors, because they keep the process of cellular progression to mitosis going on. Growth factors are also classified (along with other molecules that promote the cell cycle) as pro-mitotic signals. Hormones are also pro-mitotic signals. For example, thyrotrophic hormone, one of the hormones produced by the pituitary gland, induces the proliferation of thyroid gland's cells. Another pituitary hormone, known as growth hormone or somatotrophic hormone (STH), is responsible by body growth during childhood and early adolescence, inducing the lengthening of the long bones and **protein synthesis**. Estrogens are hormones that do not occupy a membrane receptor, but instead, penetrate the cell and the nucleus, binding directly to specific sites in the DNA, thus inducing the cell cycle.

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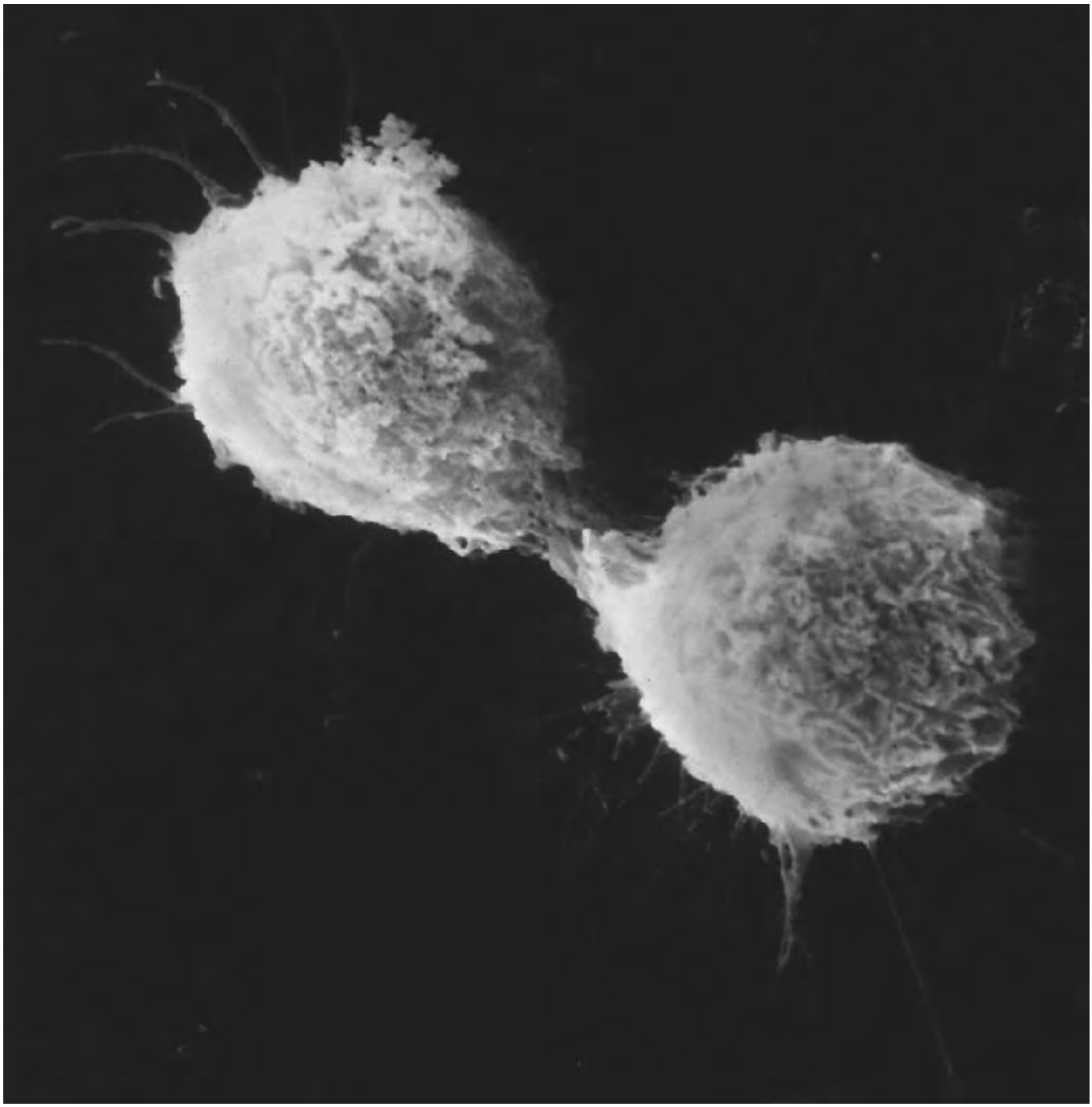
Interphase is a phase of cell growth and metabolic activity, without cell nuclear division, comprised of several stages or phases. During Gap 1 or G1 the cell resumes protein and RNA synthesis, which was interrupted during mitosis, thus allowing the growth and maturation of young cells to accomplish their physiologic function. Immediately following is a variable length pause for DNA checking and repair before cell cycle transition to phase S during which there is synthesis or semiconservative replication or synthesis of DNA. During Gap 2 or G2, there is increased RNA and protein synthesis, followed by a second pause for proofreading and eventual repairs in the newly synthesized DNA sequences before transition to Mitosis.

At the start of mitosis the chromosomes are already duplicated, with the sister-chromatids (identical chromosomes) clearly visible under a light **microscope**. Mitosis is subdivided into prophase, metaphase, anaphase and telophase.

During prophase there is a high condensation of chromatids, with the beginning of nucleolus disorganization and nuclear membrane disintegration, followed by the start of cen-



Scanning electron micrograph of eukaryotic cell division.

trioles' migration to opposite cell poles. During metaphase the chromosomes organize at the equator of a spindle apparatus (microtubules), forming a structure termed metaphase plate. The sister-chromatids are separated and joined to different centromeres, while the microtubules forming the spindle are attached to a region of the centromere termed kinetochore. During anaphase there are spindles, running from each opposite kinetochore, that pull each set of chromosomes to their respective cell poles, thus ensuring that in the following phase each new cell will ultimately receive an equal division of chromosomes. During telophase, kinetochores and spindles disintegrate, the reorganization of nucleus begins, chromatin becomes less condensed, and the nucleus membrane start forming again around each set of chromosomes. The cytoskeleton is reorganized and the somatic cell has now doubled its volume and presents two organized nucleus.

Cytokinesis usually begins during telophase, and is the process of cytoplasmatic division. This process of division

varies among species but in somatic cells, it occurs through the equal division of the cytoplasmatic content, with the plasma membrane forming inwardly a deep cleft that ultimately divides the parental cell in two new daughter cells.

The identification and detailed understanding of the many molecules involved in the cell cycle controls and intracellular signal **transduction** is presently under investigation by several research groups around the world. This knowledge is crucial to the development of new anti-cancer drugs as well as to new treatments for other genetic diseases, in which a gene over expression or deregulation may be causing either a chronic or an acute disease, or the impairment of a vital organ function. Scientists predict that the next two decades will be dedicated to the identification of gene products and their respective function in the cellular microenvironment. This new field of research is termed **proteomics**.

See also Cell cycle (Prokaryotic) genetic regulation of; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells

CELL CYCLE (PROKARYOTIC), GENETIC REGULATION OF

Although prokaryotes do not have an organized **nucleus** and other complex organelles found in eukaryotic cells, prokaryotic organisms share some common features with **eukaryotes** as far as cell division is concerned. For example, they both replicate **DNA** in a semi conservative manner, and the segregation of the newly formed DNA molecules occurs before the cell division takes place through cytokinesis. Despite such similarities, the prokaryotic genome is stored in a single DNA molecule, whereas eukaryotes may contain a varied number of DNA molecules, specific to each species, seen in the interphasic nucleus as **chromosomes**. Prokaryotic cells also differ in other ways from eukaryotic cells. Prokaryotes do not have cytoskeleton and the DNA is not condensed during mitosis. The prokaryote chromosomes do not present histones, the complexes of histonic proteins that help to pack eukaryotic DNA into a condensate state. Prokaryotic DNA has one single promoter site that initiates replication, whereas eukaryotic DNA has multiple promoter sites. Prokaryotes have a lack of spindle apparatus (or microtubules), which are essential structures for chromosome segregation in eukaryotic cells. In prokaryotes, there are no membranes and organelles dividing the cytosol in different compartments. Although two or more DNA molecules may be present in a given prokaryotic cell, they are genetically identical. They may contain one extra circular strand of genes known as plasmid, much smaller than the genomic DNA, and **plasmids** may be transferred to another prokaryote through bacterial **conjugation**, a process known as horizontal **gene** transfer.

The prokaryotic method of reproduction is asexual and is termed binary fission because one cell is divided in two new identical cells. Some prokaryotes also have a plasmid. Genes in **plasmids** are extra-chromosomal genes and can either be separately duplicated by a class of gene known as **transposons** Type II, or simply passed on to another individual. Transposons Type I may transfer and insert one or more genes from the plasmid to the cell DNA or vice-versa causing mutation through genetic **recombination**. The chromosome is attached to a region of the internal side of the membrane, forming a nucleoide. Some bacterial cells do present two or more nucleoides, but the genes they contain are identical.

The prokaryotic **cell cycle** is usually a fast process and may occur every 20 minutes in favorable conditions. However, some **bacteria**, such as *Mycobacterium leprae* (the cause of **leprosy**), take 12 days to accomplish replication in the host's leprous lesion. Replication of prokaryotic DNA, as well as of eukaryotic DNA, is a semi- conservative process, which means that each newly synthesized strand is paired with its complementary parental strand. Each daughter cell, therefore, receives a double-stranded circular DNA molecule that is formed by a new strand is paired with an old strand.

The cell cycle is regulated by genes encoding products (i.e., **enzymes** and proteins) that play crucial roles in the maintenance of an orderly sequence of events that ensures that each resultant daughter cell will inherit the same amount of genetic information. Cell induction into proliferation and DNA replication are controlled by specific gene products, such as enzyme DNA polymerase III, that binds to a promoter region in the circular DNA, initiating its replication. However, DNA polymerase requires the presence of a pre-existing strand of DNA, which serves as a template, as well as **RNA** primers, to initiate the polymerization of a new strand. Before replication starts, timidine-H^3, (a DNA precursor) is added to a Y-shaped site where the double helices were separated, known as the replicating fork. The DNA strands are separated by enzyme helicases and kept apart during replication by single strand proteins (or ss DNA-binding proteins) that binds to DNA, while the enzyme topoisomerase further unwinds and elongates the two strands to undo the circular ring.

DNA polymerase always makes the new strand by starting from the extremity 5' and terminating at the extremity 3'. Moreover, the two DNA strands have opposite directions (i.e., they keep an anti-parallel arrangement to each other). Therefore, the new strand 5' to 3' that is complementary to the old strand 3' to 5' is synthesized in a continuous process (leading strand synthesis), whereas the other new strand (3' to 5') is synthesized in several isolated fragments (lagging strand synthesis) that will be later bound together to form the whole strand. The new 3' to 5' strand is complementary to the old 5' to 3'. However, the lagging fragments, known as Okazaki's fragments, are individually synthesized in the direction 5' to 3' by DNA polymerase III. RNA polymerases produce the RNA primers that help DNA polymerases to synthesize the leading strand. Nevertheless, the small fragments of the lagging strand have as primers a special RNA that is synthesized by another enzyme, the primase. Enzyme topoisomerase III does the proofreading of the newly transcribed sequences and eliminates those wrongly transcribed, before DNA synthesis may continue. RNA primers are removed from the newly synthesized sequences by ribonuclease H. Polymerase I fills the gaps and DNA ligase joins the lagging strands.

After DNA replication, each DNA molecule is segregated, i.e., separated from the other, and attached to a different region of the internal face of the membrane. The formation of a septum, or dividing internal wall, separates the cell into halves, each containing a nucleotide. The process of splitting the cell in two identical daughter cells is known as cytokinesis.

See also Bacterial growth and division; Biochemistry; Cell cycle (eukaryotic), genetic regulation of; Cell cycle and cell division; Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); Enzymes; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Genotype and phenotype; Molecular biology and molecular genetics

CELL MEMBRANE TRANSPORT

The cell is bound by an outer membrane that, in accord with the fluid mosaic model, is comprised of a phospholipid lipid bilayer with proteins—molecules that also act as receptor sites—interspersed within the phospholipid bilayer. Varieties of channels exist within the membrane. There are a number of internal cellular membranes that partially partition the intercellular matrix, and that ultimately become continuous with the nuclear membrane.

There are three principal mechanisms of outer cellular membrane transport (i.e., means by which molecules can pass through the boundary cellular membrane). The transport mechanisms are passive, or gradient diffusion, facilitated diffusion, and active transport.

Diffusion is a process in which the random motions of molecules or other particles result in a net movement from a region of high concentration to a region of lower concentration. A familiar example of diffusion is the dissemination of floral perfumes from a bouquet to all parts of the motionless air of a room. The rate of flow of the diffusing substance is proportional to the concentration gradient for a given direction of diffusion. Thus, if the concentration of the diffusing substance is very high at the source, and is diffusing in a direction where little or none is found, the diffusion rate will be maximized. Several substances may diffuse more or less independently and simultaneously within a space or volume of liquid. Because lightweight molecules have higher average speeds than heavy molecules at the same temperature, they also tend to diffuse more rapidly. Molecules of the same weight move more rapidly at higher temperatures, increasing the rate of diffusion as the temperature rises.

Driven by concentration gradients, diffusion in the cell usually takes place through channels or pores lined by proteins. Size and electrical charge may inhibit or prohibit the passage of certain molecules or electrolytes (e.g., sodium, potassium, etc.).

Osmosis describes diffusion of water across cell membranes. Although water is a polar molecule (i.e., has overall partially positive and negative charges separated by its molecular structure), transmembrane proteins form hydrophilic (water loving) channels to through which water molecules may move.

Facilitated diffusion is the diffusion of a substance not moving against a concentration gradient (i.e., from a region of low concentration to high concentration) but which require the assistance of other molecules. These are not considered to be energetic reactions (i.e., energy in the form of use of adenosine triphosphate molecules (ATP) is not required. The facilitation or assistance—usually in physically turning or orienting a molecule so that it may more easily pass through a membrane—may be by other molecules undergoing their own random motion.

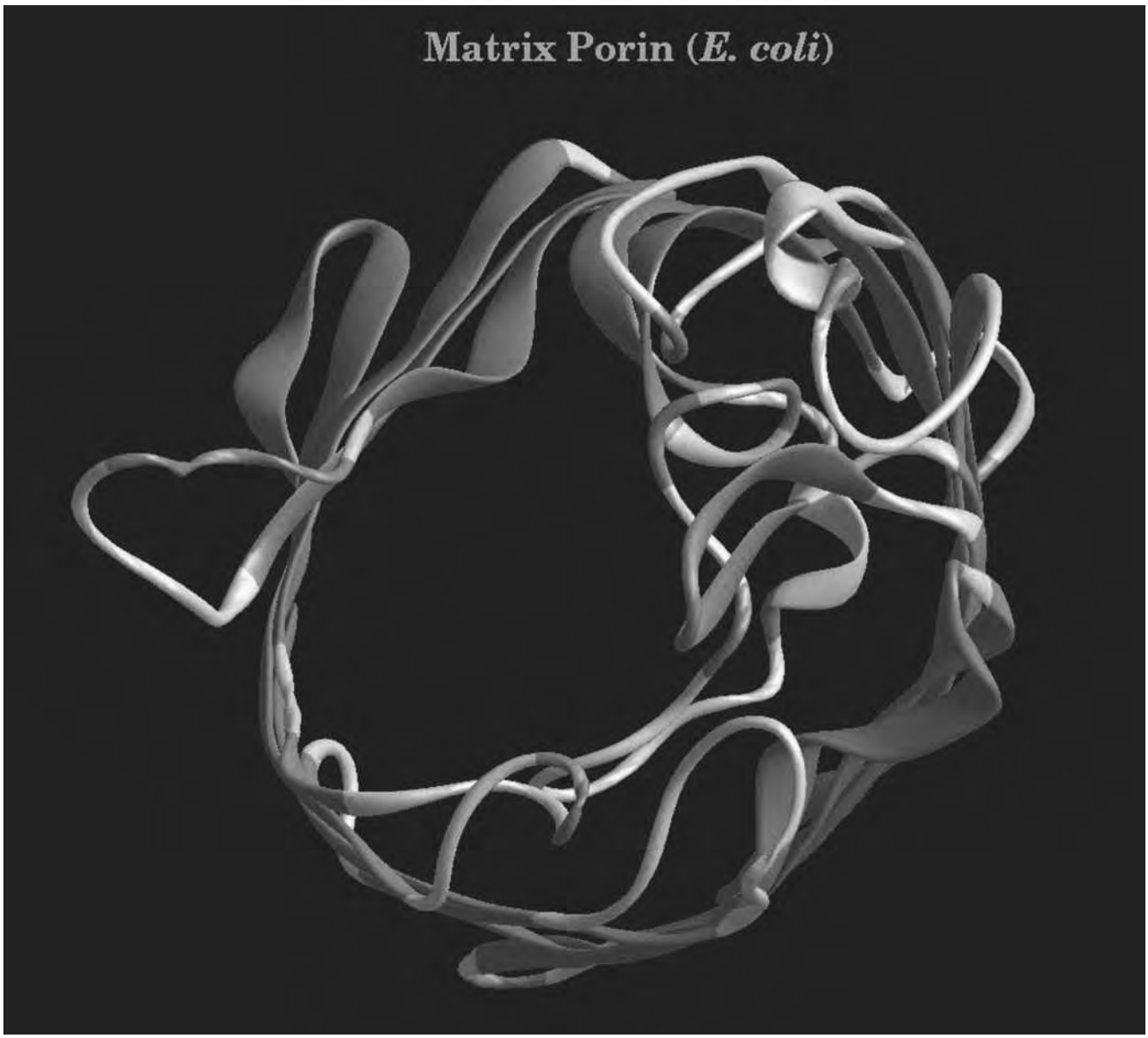
Transmembrane proteins establish pores through which ions and some small hydrophilic molecules are able to pass by diffusion. The channels open and close according to the physiological needs and state of the cell. Because they open and close transmembrane proteins are termed “gated” proteins. Control of the opening and closing mechanism may be via mechanical, electrical, or other types of membrane changes that may occur as various molecules bind to cell receptor sites.

Active transport is movement of molecules across a cell membrane or membrane of a cell organelle, from a region of low concentration to a region of high concentration. Since these molecules are being moved against a concentration gradient, cellular energy is required for active transport. Active transport allows a cell to maintain conditions different from the surrounding environment.

There are two main types of active transport; movement directly across the cell membrane with assistance from transport proteins, and endocytosis, the engulfing of materials into a cell using the processes of pinocytosis, **phagocytosis**, or receptor-mediated endocytosis.

Transport proteins found within the phospholipid bilayer of the cell membrane can move substances directly across the cell membrane, molecule by molecule. The sodium-potassium pump, which is found in many cells and helps nerve cells to pass their signals in the form of electrical impulses, is a well-studied example of active transport using transport proteins. The transport proteins that are an essential part of the sodium-potassium pump maintain a higher concentration of potassium ions inside the cells compared to outside, and a higher concentration of sodium ions outside of cells compared to inside. In order to carry the ions across the cell membrane and against the concentration gradient, the transport proteins have very specific shapes that only fit or bond well with sodium and potassium ions. Because the transport of these ions is against the concentration gradient, it requires a significant amount of energy.

Endocytosis is an infolding and then pinching in of the cell membrane so that materials are engulfed into a vacuole or vesicle within the cell. Pinocytosis is the process in which cells engulf liquids. The liquids may or may not contain dissolved materials. Phagocytosis is the process in which the materials that are taken into the cell are solid particles. With receptor-mediated endocytosis the substances that are to be transported into the cell first bind to specific sites or receptor proteins on the outside of the cell. The substances can then be engulfed into the cell. As the materials are being carried into the cell, the cell membrane pinches in forming a vacuole or other vesicle. The materials can then be used inside the cell.



View down the channel of the matrix porin of *Escherichia coli*.

Because all types of endocytosis use energy, they are considered active transport.

See also Bacterial growth and division; Biochemistry; Cell cycle and cell division; Enzymes; Molecular biology and molecular genetics

Centers for Disease Control

The Centers for Disease Control and Prevention (CDC) is one of the primary **public health** institutions in the world. CDC is headquartered in Atlanta, Georgia, with facilities at 9 other sites in the United States. The centers are the focus of the United States government efforts to develop and implement prevention and control strategies for diseases, including those of microbiological origin.

The CDC is home to 11 national centers that address various aspects of health care and disease prevention. Examples of the centers include the National Center for Chronic Disease Prevention and Health promotion, National Center for Infectious Diseases, National **Immunization** Program, and the National Center for **HIV**, STD, and TB Prevention.

CDC was originally the acronym for The Communicable Disease Center. This center was a redesignation of an existing facility known as the Malaria Control in War Areas. The malaria control effort had been mandated to eradicate

malaria from the southern United States during World War II. The Communicable Disease Center began operations in Atlanta on July 1, 1946, under the direction of Dr. Joseph M. Mountin.

Initially, the center was very small and was staffed mainly by engineers and entomologists (scientists who study insects). But under Mountin's direction, an expansion program was begun with the intent of making the center the predominant United States center of **epidemiology**. By 1950 the center had opened a disease surveillance unit that remains a cornerstone of CDC's operations today. Indeed, during the Korean War, the Epidemiological Intelligence Service was created, to protect the United States from the immigration of disease causing **microorganisms**.

Two events in the 1950s brought the CDC to national prominence and assured the ongoing funding of the center. The first event was the outbreak of **poliomyelitis** in children who had received an inoculation with the recently approved Salk polio **vaccine**. A Polio Surveillance Unit that was established at CDC confirmed the cause of the cases to be due to a contaminated batch of the vaccine. With CDC's help, the problem was solved and the national polio **vaccination** program recommenced. The other event was a massive outbreak of influenzae. Data collected by CDC helped pave the way for the development of **influenza** vaccines and inoculation programs.

In the 1950s and 1960s, CDC became the center for venereal disease, **tuberculosis**, and immunization programs. The centers also played a pivotal role in the eradication of **smallpox**, through the development of a vaccine and an inoculation instrument. Other accomplishments include the identification of **Legionnaire's disease** and **toxic shock syndrome** in the 1970s and 1980s, hantavirus pulmonary syndrome in 1993, and, beginning in 1981, a lead role in the research and treatment of Acquired **Immunodeficiency** Syndrome.

In 1961, CDC took over the task of publishing *Morbidity and Mortality Weekly Report*. Then as now, the MMWR is a definitive weekly synopsis of data on deaths and selected diseases from every state in the United States. A noteworthy publication in MMWR was the first report in a 1981 issue of the disease that would come to be known as Acquired Immunodeficiency Syndrome.

Another advance took place in 1978, with the opening of a containment facility that could be used to study the most lethal **viruses** known to exist (e.g., Ebola). Only a few such facilities exist in the world. Without such high containment facilities, hemorrhagic viruses could not be studied, and development of vaccines would be impossible.

Ultimately, CDC moved far beyond its original mandate as a communicable disease center. To reflect this change, the name of the organization was changed in 1970 to the Center for Disease Control. In 1981, the name was again changed to the Centers for Disease Control. The subsequent initiation of programs designed to target chronic diseases, breast and cervical cancers and lifestyle issues (e.g., smoking) extended CDC's mandate beyond disease control. Thus, in 1992, the organization became the Centers for Disease Control and Prevention (the acronym CDC was retained).

Today, CDC is a world renowned center of excellence for public health research, disease detection, and dissemination of information on a variety of diseases and health issues.

See also AIDS, recent advances in research and treatment; Bacteria and bacterial infection; History of public health; Public health, current issues

CEPHALOSPORINS • *see* ANTIBIOTICS

CHAGAS DISEASE

Chagas disease is a human infection that is caused by a microorganism that establishes a parasitic relationship with a human host as part of its life cycle. The disease is named for the Brazilian physician Carlos Chagas, who described in 1909 the involvement of the flagellated protozoan known as *Trypanosoma cruzi* in a prevalent disease in South America.

The disease is confined to North, South, and Central America. Reflecting this, and the similarity of the disease to trypanosomiasis, a disease that occurs on the African continent, Chagas disease has also been dubbed American trypanosomiasis. The disease affects some 16 to 18 million each year, mainly in Central and South American. Indeed, in these regions the prevalence of Chagas disease in the population is higher than that of the **Human Immunodeficiency Virus** and the **Hepatitis** B and C **viruses**. Of those who acquire Chagas disease, approximately 50,000 people die each year.

The agent of Chagas disease, *Trypanosoma cruzi*, is a member of a division, or phylum, called Sarcomastigophora. The protozoan is spread to human via a bug known as Reduviid bugs (or "kissing bugs"). These bugs are also known as triatomines. Examples of species include *Triatoma infestans*, *Triatoma brasiliensis*, *Triatoma dimidiata*, and *Triatoma sordida*.

The disease is spread because of the close proximity of the triatomine bugs and humans. The bugs inhabit houses, particularly more substandard houses where cracks and deteriorating framework allows access to interior timbers. Biting an already infected person or animal infects the bugs themselves. The protozoan lives in the digestive tract of the bug. The infected bug subsequently infects another person by defecating on them, often while the person is asleep and unaware of the bug's presence. The trypanosomes in the feces gain entry to the bloodstream when feces are accidentally rubbed into the bite, or other orifices such as the mouth or eyes.

Chagas disease can also be transmitted in the blood. Acquisition of the disease via a blood transfusion occurs in thousands of people each year.

The association between the Reduviid bug and poor quality housing tends to make Chagas disease prevalent in underdeveloped regions of Central and South America. To add to the burden of these people, some 30% of those who are infected in childhood develop a chronic form of the disease 10

to 20 years later. This long-lasting form of Chagas disease reduces the life span by almost a decade.

Chagas disease may be asymptomatic (without symptoms)—or can produce a variety of symptoms. The form of the disease that strikes soon after infection with *Trypanosoma cruzi* tends to persist only for a few months before disappearing. Usually, no treatment is necessary for relief from the infection. Symptoms of this type of so-called acute infection include swelling at the site of the bug bite, tiredness, fever, enlarged spleen or liver, diarrhea, and vomiting. Infants can experience a swelling of the brain that can be fatal.

The chronic form of Chagas disease can produce more severe symptoms, including an enlarged heart, irregularities in heart function, and the enlargement and malfunction of the digestive tract. These symptoms are of particular concern in those people whose **immune system** is not functioning properly.

Currently, there is no **vaccine** or other preventative treatment for Chagas disease. Avoidance of habitats where the Reduviid bug lives is the most prudent precaution. Unfortunately, given the economic circumstances of those most at risk, this option is not easily attainable. *Trypanosoma cruzi* can also be transmitted in the blood. Therefore, screening of blood and blood products for the presence of the protozoan is wise. Once again, however, the poverty that often plays a role in the spread of Chagas disease may also be reflected in less than adequate medical practices, including blood screening.

See also Parasites; Zoonoses

CHAIN, ERNST BORIS (1906-1979)

German–born English biochemist

Ernst Chain was instrumental in the creation of **penicillin**, the first antibiotic drug. Although the Scottish bacteriologist **Alexander Fleming** discovered the *penicillium notatum* **mold** in 1928, it was Chain who, together with **Howard Florey**, isolated the breakthrough substance that has saved countless victims of infections. For their work, Chain, Florey, and Fleming were awarded the Nobel Prize in physiology or medicine in 1945.

Chain was born in Berlin to Michael Chain and Margarete Eisner Chain. His father was a Russian immigrant who became a chemical engineer and built a successful chemical plant. The death of Michael Chain in 1919, coupled with the collapse of the post–World War I German economy, depleted the family's income so much that Margarete Chain had to open up her home as a guesthouse.

One of Chain's primary interests during his youth was music, and for a while it seemed that he would embark on a career as a concert pianist. He gave a number of recitals and for a while served as music critic for a Berlin newspaper. A cousin, whose brother–in–law had been a failed conductor, gradually convinced Chain that a career in science would be more rewarding than one in music. Although he took lessons in conducting, Chain graduated from Friedrich–Wilhelm University in 1930 with a degree in chemistry and physiology.

Chain began work at the Charite Hospital in Berlin while also conducting research at the Kaiser Wilhelm Institute for Physical Chemistry and Electrochemistry. But the increasing pressures of life in Germany, including the growing strength of the Nazi party, convinced Chain that, as a Jew, he could not expect a notable professional future in Germany. Therefore, when Hitler came to power in January 1933, Chain decided to leave. Like many others, he mistakenly believed the Nazis would soon be ousted. His mother and sister chose not to leave, and both died in concentration camps.

Chain arrived in England in April 1933, and soon acquired a position at University College Hospital Medical School. He stayed there briefly and then went to Cambridge to work under the biochemist Frederick Gowland Hopkins. Chain spent much of his time at Cambridge conducting research on **enzymes**. In 1935, Howard Florey became head of the Sir William Dunn School of Pathology at Oxford. Florey, an Australian–born pathologist, wanted a top–notch biochemist to help him with his research, and asked Hopkins for advice. Without hesitation, Hopkins suggested Chain.

Florey was actively engaged in research on the bacteriolytic substance lysozyme, which had been identified by Fleming in his quest to eradicate infection. Chain came across Fleming's reports on the penicillin mold and was immediately intrigued. He and Florey both saw great potential in the further investigation of penicillin. With the help of a Rockefeller Foundation grant, the two scientists assembled a research team and set to work on isolating the active ingredient in *Penicillium notatum*.

Fleming, who had been unable to identify the antibacterial agent in the mold, had used the mold broth itself in his experiments to kill infections. Assisted in their research by fellow scientist Norman Heatley, Chain and Florey began their work by growing large quantities of the mold in the Oxford laboratory. Once there were adequate supplies of the mold, Chain began the tedious process of isolating the "miracle" substance. Succeeding after several months in isolating small amounts of a powder that he obtained by freeze–drying the mold broth, Chain was ready for the first practical test. His experiments with laboratory mice were successful, and it was decided that more of the substance should be produced to try on humans. To do this, the scientists needed to ferment massive quantities of mold broth; it took 125 gallons of the broth to make enough penicillin powder for one tablet. By 1941, Chain and his colleagues had finally gathered enough penicillin to conduct experiments with patients. The first two of eight patients died from complications unrelated to their infections, but the remaining six, who had been on the verge of death, were completely cured.

One potential use for penicillin was the treatment of wounded soldiers, an increasingly significant issue during the Second World War. For penicillin to be widely effective, however, the researchers needed to devise a way to mass–produce the substance. Florey and Heatley went to the United States in 1941 to enlist the aid of the government and of pharmaceutical houses. New ways were found to yield more and stronger penicillin from mold broth, and by 1943, the drug went into regular medical use for Allied troops. After the war, penicillin was

made available for civilian use. The ethics of whether to make penicillin research universally available posed a particularly difficult problem for the scientific community during the war years. While some believed that the research should not be shared with the enemy, others felt that no one should be denied the benefits of penicillin. This added layers of political intrigue to the scientific pursuits of Chain and his colleagues. Even after the war, Chain experienced firsthand the results of this dilemma. As chairman of the **World Health Organization** in the late 1940s, Chain had gone to Czechoslovakia to supervise the operation of penicillin plants established there by the United Nations. He remained there until his work was done, even though the Communist coup occurred shortly after his arrival. When Chain applied for a visa to visit the United States in 1951, his request was denied by the State Department. Though no reason was given, many believed his stay in Czechoslovakia, however apolitical, was a major factor.

After the war, Chain tried to convince his colleagues that penicillin and other antibiotic research should be expanded, and he pushed for more state-of-the-art facilities at Oxford. Little came of his efforts, however, and when the Italian State Institute of Public Health in Rome offered him the opportunity to organize a biochemical and microbiological department along with a pilot plant, Chain decided to leave Oxford.

Under Chain's direction, the facilities at the State Institute became known internationally as a center for advanced research. While in Rome, Chain worked to develop new strains of penicillin and to find more efficient ways to produce the drug. Work done by a number of scientists, with Chain's guidance, yielded isolation of the basic penicillin molecule in 1958, and hundreds of new penicillin strains were soon synthesized.

In 1963, Chain was persuaded to return to England. The University of London had just established the Wolfson Laboratories at the Imperial College of Science and Technology, and Chain was asked to direct them. Through his hard work the Wolfson Laboratories earned a reputation as a first–rate research center.

In 1948, Chain had married Anne Beloff, a fellow biochemist, and in the following years she assisted him with his research. She had received her Ph.D. from Oxford and had worked at Harvard in the 1940s. The couple had three children.

Chain retired from Imperial College in 1973, but continued to lecture. He cautioned against allowing the then-new field of **molecular biology** to downplay the importance of **biochemistry** to medical research. He still played the piano, for which he had always found time even during his busiest research years. Over the years, Chain also became increasingly active in Jewish affairs. He served on the Board of Governors of the Weizmann Institute in Israel, and was an outspoken supporter of the importance of providing Jewish education for young Jewish children in England and abroad—all three of his children received part of their education in Israel.

In addition to the Nobel Prize, Chain received the Berzelius Medal in 1946, and was made a commander of the Legion d'Honneur in 1947. In 1954, he was awarded the **Paul Ehrlich** Centenary Prize. Chain was knighted by Queen Elizabeth II in 1969. Chain died of heart failure at age 73.

See also Antibiotic resistance, tests for; Bacteria and responses to bacterial infection; Chronic bacterial disease; Staphylococci and staphylococcal infections

CHAPERONES

The last two decades of the twentieth century saw the discovery of the heat-shock or cell-stress response, changes in the expression of certain proteins, and the unraveling of the function of proteins that mediate this essential cell-survival strategy. The proteins made in response to the stresses are called heat-shock proteins, stress proteins, or molecular chaperones. A large number of chaperones have been identified in **bacteria** (including archaebacteria), **yeast**, and eukaryotic cells. Fifteen different groups of proteins are now classified as chaperones. Their expression is often increased by cellular stress. Indeed, many were identified as heat-shock proteins, produced when cells were subjected to elevated temperatures. Chaperones likely function to stabilize proteins under less than ideal conditions.

The term chaperone was coined only in 1978, but the existence of chaperones is ancient, as evidenced by the conservation of the peptide sequences in the chaperones from prokaryotic and eukaryotic organisms, including humans.

Chaperones function 1) to stabilize folded proteins, 2) unfold them for translocation across membranes or for degradation, or 3) to assist in the proper folding of the proteins during assembly. These functions are vital. Accumulation of unfolded proteins due to improper functioning of chaperones can be lethal for cells. **Prions** serve as an example. Prions are an infectious agent composed solely of protein. They are present in both healthy and diseased cells. The difference is that in diseased cells the folding of the protein is different. Accumulation of the misfolded proteins in brain tissue kills nerve cells. The result for the affected individual can be dementia and death, as in the conditions of kuru, Creutzfeld-Jakob disease and "mad cow" disease (bovine spongiform encephalopthy).

Chaperones share several common features. They interact with unfolded or partially folded protein subunits, nascent chains emerging from the ribosome, or extended chains being translocated across subcellular membranes. They do not, however, form part of the final folded protein molecule. Chaperones often facilitate the coupling of cellular energy sources (adenosine triphosphate; ATP) to the folding process. Finally, chaperones are essential for viability.

Chaperones differ in that some are non-specific, interacting with a wide variety of polypeptide chains, while others are restricted to specific targets. Another difference concerns their shape; some are donut-like, with the central zone as the direct interaction region, while others are block-like, tunnel-like, or consist of paired subunits.

The reason for chaperone's importance lies with the environment within cells. Cells have a watery environment, yet many of the amino acids in a protein are **hydrophobic**

(water hating). These are hidden in the interior of a correctly folded protein, exposing the hydrophilic (water loving) amino acids to the watery interior solution of the cell. If folded in such a correct manner, tensions are minimized and the three-dimensional structure of the protein is stable. Chaperons function to aid the folding process, ensuring protein stability and proper function.

Protein folding occurs by trial and error. If the protein folds the wrong way, it is captured by a chaperone, and another attempt at folding can occur. Even correctly folded proteins are subject to external stress that can disrupt structure. The chaperones, which are produced in greater amounts when a cell is exposed to higher temperatures, function to stabilize the unraveling proteins until the environmental crisis passes.

Non-biological molecules can also participate as chaperones. In this category, protein folding can be increased by the addition of agents such as glycerol, guanidium chloride, urea, and sodium chloride. Folding is likely due to an electrostatic interaction between exposed charged groups on the unfolded protein and the anions.

Increasing attention is being paid to the potential roles of chaperones in human diseases, including infection and idiopathic conditions such as arthritis and atherosclerosis. One subgroup of chaperones, the chaperonins, has received the most attention in this regard, because, in addition to facilitating protein folding, they also act as cell-to-cell signaling molecules.

See also Proteins and enzymes

CHASE, MARTHA COWLES (1927-)

American geneticist

Martha Cowles Chase is remembered for a landmark experiment in genetics carried out with American geneticist **Alfred Day Hershey** (1908–1997). Their experiment indicated that, contrary to prevailing opinion in 1952, **DNA** was genetic material. A year later, **James D. Watson** and British biophysicist **Francis Crick** proposed their double helical model for the three-dimensional structure of structure of DNA. Hershey was honored as one of the founders of **molecular biology**, and shared the 1969 Nobel Prize in medicine or physiology with Salvador Luria and Max Delbrück.

Martha Chase was born in Cleveland, Ohio. She earned a bachelor's degree from the College of Wooster in 1950 and her doctoral degree from the University of Southern California in 1964. Having married and changed her name to Martha C. Epstein (Martha Cowles Chase Epstein), she later returned to Cleveland Heights, Ohio, where she lived with her father, Samuel W. Chase. After graduating from college, Chase worked as an assistant to Alfred Hershey at the Carnegie Institution of Washington in Cold Spring Harbor, New York. This was a critical period in the history of modern genetics and the beginning of an entirely new phase of research that established the science of molecular biology. Including the name of an assistant or technician on a publication, especially one that was certain to become a landmark in the history of molecular biology, was unusual during the 1960s. Thus, it is remarkable that Martha Chase's name is inextricably linked to all accounts of the path to the demonstration that DNA is the genetic material.

During the 1940s, most chemists, physicists, and geneticists thought that the genetic material must be a protein, but research on the **bacteria** that cause **pneumonia** suggested the nucleic acids played a fundamental role in inheritance. The first well-known series of experiments to challenge the assumption that genes must be proteins or nucleoproteins was carried out by **Oswald T. Avery** (1877–1955) and his co-workers **Colin Macleod**, and **Maclyn McCarty** in 1944. Avery's work was a refinement of observations previously reported in 1928 by Fred Griffith (1877–1941), a British bacteriologist. Avery identified the transforming principle of bacterial types as DNA and noted that further studies of the chemistry of DNA were required in order to explain its biological activity.

Most geneticists were skeptical about the possibility that DNA could serve as the genetic material until the results of the Hershey-Chase experiments of 1952 were reported. Their experiments indicated that bacteriophages (**viruses** that attack bacteria) might act like tiny syringes containing the genetic material and the empty virus containers might remain outside the bacterial cell after the genetic material of the virus had been injected. To test this possibility, Hershey and Chase used radioactive sulfur to label **bacteriophage** proteins and radioactive phosphate to label their DNA. After allowing viruses to attack the bacterial cells, the bacterial cultures were spun in a blender and centrifuged in order to separate intact bacteria from smaller particles.

Hershey and Chase found that most of the bacteriophage DNA remained with the bacterial cells while their protein coats were released into the medium. They concluded that the protein played a role in adsorption to the bacteria and helped inject the viral DNA into the bacterial cell. Thus, it was the DNA that was involved in the growth and multiplication of bacteriophage within the infected bacterial cell. Friends of Alfred Hershey recalled that when he was asked for his concept of the greatest scientific happiness, he said it would be to have an experiment that works. The Hershey-Chase experiments became a proverbial example of what his friends and colleagues called "Hershey Heaven."

See also Bacteriophage and bacteriophage typing; DNA (Deoxyribonucleic acid); Molecular biology and molecular genetics; Molecular biology, central dogma of; Viral genetics

CHEMICAL MUTAGENESIS

The interaction of certain environmental chemical compounds and cell **metabolism** may result in genetic changes in **DNA** structure, affecting one or more genes. These chemical-induced **mutations** are known as chemical mutagenesis. Many cancers and other degenerative diseases result from acquired genetic mutations due to environmental exposure, and not as an outcome of inherited traits. Chemicals capable of inducing genetic mutation (i.e., chemical mutagenes or genotoxic compounds) are present in both natural and man-made environments and products.

Many plants, including edible ones, produce discreet amounts of some toxic compound that plays a role in plant protection against some natural predator. Some of these natural compounds may also be genotoxic for humans and animals, when that plant is consumed frequently and in great amounts. For instance, most edible mushrooms contain a family of chemical mutagenes known as hydrazines; but once mushrooms are cooked, most hydrazines evaporate or are degraded into less toxic compounds.

Among the most aggressive man-made chemical mutagenes are:

- asbestos
- DDT
- insecticides and herbicides containing arsenic
- industrial products containing benzene
- formaldehyde
- diesel and gasoline exhaust
- polychlorinated biphenyl (PCB)

Exposure to some of these compounds may occur in the work place, others can be present in the polluted air of great cities and industrial districts. For instance, insecticide and herbicide sprayers on farms, tanners, and oil refinery workers are frequently exposed to arsenic and may suffer mutations that lead to lung or skin cancers. Insulation and demolition workers are prone to **contamination** with asbestos and may eventually develop lung cancer. Painters, dye users, furniture finishers, and rubber workers are often exposed to benzene, which can induce mutations in stem cells that generate white blood cells, thus causing myelogenous leukemia. People working in the manufacture of wood products, paper, textiles and metallurgy, as well as hospital and laboratory workers, are frequently in contact with formaldehyde and can thus suffer mutations leading to nose and nasopharynx tumors. Cigarette and cigar smoke contains a class of chemical mutagenes, known as PAH (polycyclic aromatic hydrocarbons), that leads to mutation in lung cells. PAH is also present in gas and diesel combustion fumes.

Except for the cases of accidental high exposure and contamination, most chemical mutagenes or their metabolites (i.e., cell-transformed by-products) have a progressive and gradual accumulation in DNA, throughout years of exposition. Some individuals are more susceptible to the effects of cumulative contamination than others. Such individual degrees of susceptibility are due to discreet genetic variations, known as polymorphism, meaning several forms or versions of a given group of genes. Depending on the polymorphic version of Cytochrome P450 genes, an individual may metabolize some mutagenes faster than others. Polymorphism in another group of genes, NAT (N-acetyltransferase), is also implied in different individual susceptibilities to chemical exposure and mutagenesis.

See also Immunogenetics; Mutants, enhanced tolerance or sensitivity to temperature and pH ranges; Mutations and mutagenesis

CHEMOAUTOTROPHIC AND CHEMOLITHOTROPHIC BACTERIA

Autotrophic bacteria obtain the carbon that they need to sustain survival and growth from carbon dioxide (CO_2). To process this carbon source, the **bacteria** require energy. Chemoautotrophic bacteria and chemolithotrophic bacteria obtain their energy from the oxidation of inorganic (non-carbon) compounds. That is, they derive their energy from the energy already stored in chemical compounds. By oxidizing the compounds, the energy stored in chemical bonds can be utilized in cellular processes. Examples of inorganic compounds that are used by these types of bacteria are sulfur, ammonium ion (NH^{4+}), and ferrous iron (Fe^{2+}).

The designation autotroph means "self nourishing." Indeed, both chemoautotrophs and chemolithotrophs are able to grow on medium that is free of carbon. The designation lithotrophic means "rock eating," further attesting to the ability of these bacteria to grow in seemingly inhospitable environments.

Most bacteria are chemotrophic. If the energy source consists of large chemicals that are complex in structure, as is the case when the chemicals are derived from once-living organisms, then it is the chemoautotrophic bacteria that utilize the source. If the molecules are small, as with the elements listed above, they can be utilized by chemolithotrophs.

Only bacteria are chemolithotrophs. Chemoautotrophs include bacteria, **fungi**, animals, and **protozoa**.

There are several common groups of chemoautotrophic bacteria. The first group is the colorless sulfur bacteria. These bacteria are distinct from the sulfur bacteria that utilize sunlight. The latter contain the compound **chlorophyll**, and so appear colored. Colorless sulfur bacteria oxidize hydrogen sulfide (H_2S) by accepting an electron from the compound. The acceptance of an electron by an oxygen atom creates water and sulfur. The energy from this reaction is then used to reduce carbon dioxide to create carbohydrates. An example of a colorless sulfur bacteria is the genus *Thiothrix*.

Another type of chemoautotroph is the "iron" bacteria. These bacteria are most commonly encountered as the rusty coloured and slimy layer that builds up on the inside of toilet tanks. In a series of chemical reactions that is similar to those of the sulfur bacteria, iron bacteria oxidize iron compounds and use the energy gained from this reaction to drive the formation of carbohydrates. Examples of iron bacteria are *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. These bacteria are common in the runoff from coal mines. The water is very acidic and contains ferrous iron. Chemoautotrophs thrive in such an environment.

A third type of chemoautotrophic bacteria includes the nitrifying bacteria. These chemoautotrophs oxidize ammonia (NH_3) to nitrate (NO_3^-). Plants can use the nitrate as a nutrient source. These nitrifying bacteria are important in the operation of the global nitrogen cycle. Examples of chemoautotrophic nitrifying bacteria include Nitrosomonas and Nitrobacter.

The **evolution** of bacteria to exist as chemoautotrophs or chemolithotrophs has allowed them to occupy niches that

would otherwise be devoid of bacterial life. For example, in recent years scientists have studied a cave near Lovell, Wyoming. The groundwater running through the cave contains a strong sulfuric acid. Moreover, there is no sunlight. The only source of life for the thriving bacterial populations that adhere to the rocks are the rocks and the chemistry of the groundwater.

The energy yield from the use of inorganic compounds is not nearly as great as the energy that can be obtained by other types of bacteria. But, chemoautotrophs and chemolithotrophs do not usually face competition from other **microorganisms**, so the energy they are able to obtain is sufficient to sustain their existence. Indeed, the inorganic processes associated with chemoautotrophs and chemolithotrophs may make these bacteria one of the most important sources of weathering and erosion of rocks on Earth.

The ability of chemoautotrophic and chemolithotrophic bacteria to thrive through the energy gained by inorganic processes is the basis for the metabolic activities of the so-called **extremophiles**. These are bacteria that live in extremes of **pH**, temperature of pressure, as three examples. Moreover, it has been suggested that the metabolic capabilities of extremophiles could be duplicated on extraterrestrial planetary bodies.

See also Metabolism

CHEMOSTAT AND TURBIDOSTAT • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

CHEMOTAXIS • *see* BACTERIAL MOVEMENT

CHEMOTHERAPY

Chemotherapy is the treatment of a disease or condition with chemicals that have a specific effect on its cause, such as a microorganism or cancer cell. The first modern therapeutic chemical was derived from a synthetic dye. The sulfonamide drugs developed in the 1930s, **penicillin** and other **antibiotics** of the 1940s, hormones in the 1950s, and more recent drugs that interfere with cancer cell **metabolism** and reproduction have all been part of the chemotherapeutic arsenal.

The first drug to treat widespread **bacteria** was developed in the mid-1930s by the German physician-chemist Gerhard Domagk. In 1932, he discovered that a dye named prontosil killed streptococcus bacteria, and it was quickly used medically on both streptococcus and staphylococcus. One of the first patients cured with it was Domagk's own daughter. In 1936, the Swiss biochemist Daniele Bovet, working at the Pasteur Institute in Paris, showed that only a part of prontosil was active, a sulfonamide radical long known to chemists. Because it was much less expensive to produce, sulfonamide soon became the basis for several widely used "sulfa drugs" that revolutionized the treatment of formerly fatal diseases. These included **pneumonia**, **meningitis**, and puerperal ("childbed") fever. For his work, Domagk received the 1939 Nobel Prize in physiology or medicine. Though largely replaced by antibiotics, **sulfa drugs** are still commonly used against urinary tract infections, Hanson disease (**leprosy**), **malaria**, and for burn treatment.

At the same time, the next breakthrough in chemotherapy, penicillin, was in the wings. In 1928, the British bacteriologist **Alexander Fleming** noticed that a **mold** on an uncovered laboratory dish of staphylococcus destroyed the bacteria. He identified the mold as *Penicillium notatum,* which was related to ordinary bread mold. Fleming named the mold's active substance penicillin, but was unable to isolate it.

In 1939, the American microbiologist **René Jules Dubos** (1901–1982) isolated from a soil microorganism an antibacterial substance that he named tyrothricin. This led to wide interest in penicillin, which was isolated in 1941 by two biochemists at Oxford University, **Howard Florey** and **Ernst Chain**.

The term antibiotic was coined by American microbiologist **Selman Abraham Waksman**, who discovered the first antibiotic that was effective on gram-negative bacteria. Isolating it from a Streptomyces fungus that he had studied for decades, Waksman named his antibiotic streptomycin. Though streptomycin occasionally resulted in unwanted side effects, it paved the way for the discovery of other antibiotics. The first of the tetracyclines was discovered in 1948 by the American botanist Benjamin Minge Duggar. Working with *Streptomyces aureofaciens* at the Lederle division of the American Cyanamid Co., Duggar discovered chlortetracycline (Aureomycin).

The first effective chemotherapeutic agent against **viruses** was acyclovir, produced in the early 1950s by the American biochemists George Hitchings and **Gertrude Belle Elion** for the treatment of **herpes**. Today's **antiviral drugs** are being used to inhibit the reproductive cycle of both **DNA** and **RNA** viruses. For example, two drugs are used against the **influenza** A virus, Amantadine and Rimantadine, and the **AIDS** treatment drug AZT inhibits the reproduction of the **human immunodeficiency virus** (**HIV**).

Cancer treatment scientists began trying various chemical compounds for use as cancer treatments as early as the mid-nineteenth century. But the first effective treatments were the sex hormones, first used in 1945, estrogens for prostate cancer and both estrogens and androgens to treat breast cancer. In 1946, the American scientist Cornelius Rhoads developed the first drug especially for cancer treatment. It was an alkylating compound, derived from the chemical warfare agent nitrogen mustard, which binds with chemical groups in the cell's DNA, keeping it from reproducing. Alkylating compounds are still important in cancer treatment.

In the next twenty years, scientists developed a series of useful antineoplastic (anti-cancer) drugs, and, in 1954, the forerunner of the National Cancer Institute was established in Bethesda, MD. Leading the research efforts were the so-called "4-H Club" of cancer chemotherapy: the Americans Charles Huggins (1901–1997), who worked with hormones; George Hitchings (1905–1998), purines and pyrimidines to interfere with cell metabolism; Charles Heidelberger, fluorinated compounds; and British scientist Alexander Haddow (1907–1976),

who worked with various substances. The first widely used drug was 6-Mercaptopurine, synthesized by Elion and Hitchings in 1952.

Chemotherapy is used alone, in combination, and along with radiation and/or surgery, with varying success rates, depending on the type of cancer and whether it is localized or has spread to other parts of the body. They are also used after treatment to keep the cancer from recurring (**adjuvant** therapy). Since many of the drugs have severe side effects, their value must always be weighed against the serious short-and long-term effects, particularly in children, whose bodies are still growing and developing.

In addition to the male and female sex hormones androgen, estrogen, and progestins, scientists also use the hormone somatostatin, which inhibits production of growth hormone and growth factors. They also use substances that inhibit the action of the body's own hormones. An example is Tamoxifen, used against breast cancer. Normally the body's own estrogen causes growth of breast tissues, including the cancer. The drug binds to cell receptors instead, causing reduction of tissue and cancer cell size.

Forms of the B-vitamin folic acid were found to be useful in disrupting cancer cell metabolism by the American scientist Sidney Farber (1903–1973) in 1948. Today they are used on leukemia, breast cancer, and other cancers.

Plant alkaloids have long been used as medicines, such as colchicine from the autumn crocus. Cancer therapy drugs include vincristine and vinblastine, derived from the pink periwinkle by American Irving S. Johnson (1925–). They prevent mitosis (division) in cancer cells. VP-16 and VM-16 are derived from the roots and rhizomes of the may apple or mandrake plant, and are used to treat various cancers. Taxol, which is derived from the bark of several species of yew trees, was discovered in 1978, and is used for treatment of ovarian and breast cancer.

Another class of naturally occurring substances are anthracyclines, which scientists consider to be extremely useful against breast, lung, thyroid, stomach, and other cancers.

Certain antibiotics are also effective against cancer cells by binding to DNA and inhibiting RNA and **protein synthesis**. Actinomycin D, derived from Streptomyces, was discovered by Selman Waksman and first used in 1965 by American researcher Seymour Farber. It is now used against cancer of female reproductive organs, brain tumors, and other cancers.

A form of the metal platinum called cisplatin stops cancer cells' division and disrupts their growth pattern. Newer treatments that are biological or based on proteins or genetic material and can target specific cells are also being developed. Monoclonal antibodies are genetically engineered copies of proteins used by the **immune system** to fight disease. Rituximab was the first moncoclonal **antibody** approved for use in cancer, and more are under development. **Interferons** are proteins released by cells when invaded by a virus. Interferons serve to alert the body's immune system of an impending attack, thus causing the production of other proteins that fight off disease. Interferons are being studied for treating a number of cancers, including a form of skin cancer called multiple myeloma. A third group of drugs are called anti-sense drugs, which affect specific genes within cells. Made of genetic material that binds with and neutralizes messenger-RNA, anti-sense drugs halt the production of proteins within the cancer cell.

Genetically engineered cancer vaccines are also being tested against several virus-related cancers, including liver, cervix, nose and throat, kidney, lung, and prostate cancers. The primary goal of genetically engineered vaccines is to trigger the body's immune system to produce more cells that will react to and kill cancer cells. One approach involves isolating white blood cells that will kill cancer and then to find certain antigens, or proteins, that can be taken from these cells and injected into the patient to spur on the immune system. A "vaccine **gene** gun" has also been developed to inject DNA directly into the tumor cell. An RNA cancer **vaccine** is also being tested. Unlike most vaccines, which have been primarily tailored for specific patients and cancers, the RNA cancer vaccine is designed to treat a broad number of cancers in many patients.

As research into cancer treatment continues, new cancer-fighting drugs will continue to become part of the medical armamentarium. Many of these drugs will come from the burgeoning **biotechnology** industry and promise to have fewer side effects than traditional chemotherapy and radiation.

See also Antibiotic resistance, tests for; Antiviral drugs; Bacteria and bacterial infection; Blood borne infections; Cell cycle and cell division; Germ theory of disease; History of microbiology; History of public health; Immunization

CHICKEN POX • *see* ANTIBIOTICS

CHITIN

Chitin is a polymer, a repeating arrangement of a chemical structure. Chitin is found in the supporting structures of many organisms. Of relevance to microbiology, chitin is present in fungal species such as mushrooms, where it can comprise from 5% to 20% of the weight of the organism.

The backbone of chitin is a six-member carbon ring that has side groups attached to some of the carbon atoms. This structure is very similar to that of cellulose. One of the side groups of chitin is known as acetamide, whereas cellulose has hydroxy (OH) side groups.

Chitin is a noteworthy biological feature because it is constructed solely from materials that are naturally available. In contrast, most polymers are man-made and are comprised of constituents that must be artificially manufactured.

The purpose of chitin is to provide support for the organism. The degree of support depends on the amount and the thickness of chitin that is present. In **fungi** such as mushrooms, chitin confers stability and rigidity, yet allows some flexibility. This allows the mushrooms to stand and still be flexible enough to sway without snapping.

The role of chitin as a support structure is analogous to the **peptidoglycan** supportive layer that is a feature of Gram-positive and Gram-negative **bacteria**. The think peptidoglycan layer in Gram-positive bacteria provides a rigid and robust support. The peptidoglycan layer in Gram-negative bacteria that is only one molecule thick does not provide the same degree of structural support. Other mechanical elements of the Gram-negative cell wall are necessary to shore up the structure.

In the ocean, where many creatures contain chitin, sea-dwelling bacteria called *Vibrio furnisii* have evolved a sensory system that detects discarded chitin. The bacteria are able to break down the polymer and use the sugar molecules as metabolic fuel.

See also Fungi

CHLAMYDIAL PNEUMONIA

Chlamydial **pneumonia** is a pneumonia cause by one of several forms of Chlamydial **bacteria**. The three major forms of *Chlamydia* responsible for pneumonia are *Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Chlamydia trachomatis*.

In reaction to infection, infected lung tissue may become obstructed with secretions. As part of a generalized swelling or **inflammation** of the lungs, the fluid or pus secretions block the normal vascular exchanges that take place in the alveolar air sacs. Blockage of the alveoli results in a decreased oxygenation of the blood and deprivation of oxygen to tissues.

Chlamydia pneumoniae (in older literature known as "Taiwan acute respiratory agent") usually produces a condition known as "walking pneumonia," a milder form of pneumonia that may only result in a fever and persistent cough. Although the symptoms are usually mild, they can be debilitating and dangerous to at risk groups that include the elderly, young children, or to individuals already weakened by another illness. *Chlamydia pneumoniae* spreads easily and the high transmission rate means that many individuals within a population—including at risk individuals can be rapidly exposed.

Species of chlamydiae can be directly detected following cultivation in embryonated egg cultures and **immunofluorescence** staining or via **polymerase chain reaction** (**PCR**). Chlamydiae can also be detected via specific serologic tests.

Chlamydia psittaci is an avian bacteria that is transmitted by human contact with infected birds, feathers from infected birds, or droppings from infected birds. The specific pneumonia (psittacosis) may be severe and last for several weeks. The pneumonia is generally more dangerous than the form caused by *Chlamydia pneumoniae*.

Chlamydia trachomatis is the underlying bacterium responsible for one of several types of **sexually transmitted diseases** (STD). Most frequently *Chlamydia trachomatis* results in an inflammation of the urethra (nongonococcal urethritis) and pelvic inflammatory disease. Active *Chlamydia trachomatis* infections are especially dangerous during pregnancy because the newborn may come in contact with the bacteria in the vaginal canal and aspirate the bacteria into its lung tissue from coating left on the mouth and nose. Although many newborns develop only mild pneumonia, because the lungs of a newborn are fragile, especially in pre-term babies, any infection of lung tissue is serious and can be life-threatening.

Specific **antibiotics** are used to fight chlamydial pneumonias. Erythromycin and erythromycin derivatives are used to combat *Chlamydia pneumoniae* and *Chlamydia trachomatis*. Tetracycline is usually effective against *Chlamydia psittaci*.

See also Bacteria and bacterial infection; Transmission of pathogens

CHLORAMPHENICOL • *see* ANTIBIOTICS

CHLORINATION

Chlorination refers to a chemical process that is used primarily to disinfect drinking water and spills of **microorganisms**. The active agent in chlorination is the element chlorine, or a derivative of chlorine (e.g., chlorine dioxide). Chlorination is a swift and economical means of destroying many, but not all, microorganisms that are a health-threat in fluid such as drinking water.

Chlorine is widely popular for this application because of its ability to kill **bacteria** and other disease-causing organisms at relatively low concentrations and with little risk to humans. The killing effect occurs in seconds. Much of the killing effect in bacteria is due to the binding of chlorine to reactive groups within the membrane(s) of the bacteria. This binding destabilizes the membrane, leading to the explosive death of the bacterium. As well, chlorine inhibits various biochemical reactions in the bacterium. In contrast to the rapid action of chlorine, other water **disinfection** methods, such as the use of ozone or ultraviolet light, require minutes of exposure to a microorganism to kill the organism.

In many water treatment facilities, chlorine gas is pumped directly into water until it reaches a concentration that is determined to kill microorganisms, while at the same time not imparting a foul taste or odor to the water. The exact concentration depends on the original purity of the water supply. For example, surface waters contain more organic material that acts to absorb the added chlorine. Thus, more chlorine needs to be added to this water than to water emerging from deep underground. For a particular treatment facility, the amount of chlorine that is effective is determined by monitoring the water for the amount of chlorine remaining in solution and for so-called indictor microorganisms (e.g., ***Escherichia coli***).

Alternatively, chlorine can be added to water in the form of a solid compound (e.g., calcium or sodium hypochlorite). Both of these compounds react with water, releasing free chlorine. Both methods of chlorination are so inexpensive that nearly every public water purification system in the world has adopted one or the other as its primary means of destroying disease-causing organisms.

Despite this popularity, chlorination is not without drawbacks. Microorganisms such as *Cryptosporidium* and *Giardia* form dormant structures called cysts that are resistant to chlorination. The prevalence of these protozoans in worldwide drinking water supplies is increasing. Thus, the effectiveness of chlorination may be compromised in some water systems. As well, adherent bacterial populations of bacteria such as *Escherichia coli* that form in distribution pipelines are extremely resistant to chlorine, and so can contaminate the disinfected water that flows from the treatment plant to the tap. A third concern with chlorination is the reaction between chlorine and methane gas, which produces one or more chlorinated derivatives. The best known are trichloromethane (chloroform) and tetrachloromethane (carbon tetrachloride). These chlorinated hydrocarbons have been shown to have adverse health effects in humans when ingested in sufficient quantity for a long time.

Furthermore, from an engineering point of view, excess chlorine can be corrosive to pipelines. In older water treatment systems in the United States, for example, the deterioration of the water distribution pipelines is a significant problem to water delivery and **water quality**.

See also Infection control; Water quality

CHLOROPHYLL

Chlorophyll is a green pigment contained in the foliage of plants, giving them their notable coloration. This pigment is responsible for absorbing sunlight required for the production of sugar molecules, and ultimately of all biochemicals, in the plant.

Chlorophyll is found in the thylakoid sacs of the **chloroplast**. The chloroplast is a specialized part of the cell that functions as an organelle. Once the appropriate wavelengths of light are absorbed by the chlorophyll into the thylakoid sacs, the important process of **photosynthesis** is able to begin. In photosynthesis, the chloroplast absorbs light energy, and converts it into the chemical energy of simple sugars.

Vascular plants, which can absorb and conduct moisture and nutrients through specialized systems, have two different types of chlorophyll. The two types of chlorophyll, designated as chlorophyll a and b, differ slightly in chemical makeup and in color. These chlorophyll molecules are associated with specialized proteins that are able to penetrate into or span the membrane of the thylakoid sac.

When a chlorophyll molecule absorbs light energy, it becomes an excited state, which allows the initial chain reaction of photosynthesis to occur. The pigment molecules cluster together in what is called a photosynthetic unit. Several hundred chlorophyll a and chlorophyll b molecules are found in one photosynthetic unit.

A photosynthetic unit absorbs light energy. Red and blue wavelengths of light are absorbed. Green light cannot be absorbed by the chlorophyll and the light is reflected, making the plant appear green. Once the light energy penetrates these pigment molecules, the energy is passed to one chlorophyll molecule, called the reaction center chlorophyll. When this molecule becomes excited, the light reactions of photosynthesis can proceed. With carbon dioxide, water, and the help of specialized **enzymes**, the light energy absorbed creates chemical energy in a form the cell can use to carry on its processes.

In addition to chlorophyll, there are other pigments known as accessory pigments that are able to absorb light where the chlorophyll is unable to. Carotenoids, like B-carotenoid, are also located in the thylakoid membrane. Carotenoids give carrots and some autumn leaves their color. Several different pigments are found in the chloroplasts of algae, **bacteria**, and **diatoms**, coloring them varying shades of red, orange, blue, and violet.

See also Autotrophic bacteria; Blue-green algae

CHLOROPHYTA

Chlorophyta are **microorganisms** that are grouped in the kingdom called Protista. The microbes are plant-like, in that they are able to manufacture energy from sunlight. The microbes are also commonly known as green algae

Depending on the species, Chlorophyta can be single-celled, multicelled, and can associate together in colonies. The environmental diversity of Chlorophyta is vast. Many types live in marine and fresh water. Terrestrial habitats include tree trunks, moist rocks, snowbanks, and creatures including turtles, sloths and mollusks. There are some 8,000 species of chlorophytes, ranging in size from microscopic to visibly large.

There are three classes of Chlorophyta. The first class, which contains the greatest number of organisms, is called Chlorophyceae. A notable example of an organism from this class is Chlorella, which is economically important as a dietary supplement. Another member of the class is *Volvox*, a spherical organized community containing upwards of 60,000 cells.

The second class is called Charophyceae. Members of this class have existed since prehistoric times, as evidenced by fossil finds. An example of this class is *Spirogyra*, which form slimy filaments on the surface of freshwater.

The third class is called Ulvophyceae. These are marine organisms. Some become associated with sea slugs where they provide the slug with oxygen and are in turn provided with protection and nutrients. Species of a calcium-rich green algae called *Halimeda* form the blinding white sand beaches of the Caribbean when they wash up onshore and become bleached by the sun. Another example from this class is *Ulva* that grows on rocks and wharves as green, leafy-appearing clusters.

Chlorophyta contain structures that are called chloroplasts. Within the chloroplasts two pigments (**chlorophyll** a and chlorophyll b) are responsible for the conversion of sunlight to chemical energy. The energy is typically stored as starch, and in their cell walls, which are composed of a material called cellulose. The stored material can be used for energy as needed. This process of energy generation is similar to that which occurs in plants. There is an evolutionary basis for this similarity. Available evidence indicates that members of Chlorophyta were the precursors of plants. Chlorophyte

Thin section electron micrograph showing the stacked arrangement of chloroplast membranes.

fossils date from over one billion years ago, before the development of plants.

See also Photosynthesis

CHLOROPLAST

Chloroplasts are organelles—specialized parts of a cell that function in an organ-like fashion. They are found in vascular plants, mosses, liverworts, and algae. Chloroplast organelles are responsible for **photosynthesis**, the process by which sunlight is absorbed and converted into fixed chemical energy in the form of simple sugars synthesized from carbon dioxide and water.

Chloroplasts are located in the mesophyll, a green tissue area in plant leaves. Four layers or zones define the structure of a chloroplast. The chloroplast is a small lens-shaped organelle that is enclosed by two membranes with a narrow intermembrane space, known as the chloroplast envelope. Raw material and products for photosynthesis enter in and pass out through this double membrane, the first layer of the structure.

Inside the chloroplast envelope is the second layer, which is an area filled with a fluid called stroma. A series of chemical reactions involving **enzymes** and the incorporation of carbon dioxide into organic compounds occur in this region.

The third layer is a membrane-like structure of thylakoid sacs. Stacked like poker chips, the thylakoid sacs form a grana. These grana stacks are connected by membranous structures. Thylakoid sacs contain a green pigment called **chlorophyll**. In this region the thylakoid sacs, or grana, absorb light energy using this pigment. Chlorophyll absorbs light between the red and blue spectrums and reflects green light, making leaves appear green. Once the light energy is absorbed into the final layer, the intrathylakoid sac, the important process of photosynthesis can begin.

Scientists have attempted to discover how chloroplasts convert light energy to the chemical energy stored in organic molecules for a long time. It has only been since the beginning of this century that scientists have begun to understand this process. The following equation is a simple formula for photosynthesis:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2.$$

Carbon dioxide plus water produce a carbohydrate plus oxygen. Simply, this means that the chloroplast is able to split water into hydrogen and oxygen.

Many questions still remain unanswered about the complete process and role of the chloroplast. Researchers continue to study the chloroplast and its **evolution**. Based on studies of the evolution of early complex cells, scientists have devised the serial endosymbiosis theory. It is suspected that primitive microbes were able to evolve into more complex microbes by incorporating other photosynthetic microbes into their cellular structures and allowing them to continue functioning as organelles. As **molecular biology** becomes more sophisticated, the origin and genetic makeup of the chloroplast will be more clearly understood.

See also Autotrophic bacteria; Blue-green algae; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses

CHROMOSOMES, EUKARYOTIC

Chromosomes are microscopic units containing organized genetic information, eukaryotic chromosomes are located in the nuclei of diploid and haploid cells (e.g., human somatic and sex cells). Prokaryotic chromosomes are also present in one-cell non-nucleated (unicellular **microorganisms**) prokaryotic cells (e.g., **bacteria**). The sum-total of genetic information contained in different chromosomes of a given individual or species are generically referred to as the genome.

In humans, eukaryotic chromosomes are structurally made of roughly equal amounts of proteins and **DNA**. Each chromosome contains a double-strand DNA molecule, arranged as a double helix, and tightly coiled and neatly packed by a family of proteins called histones. DNA strands are comprised of linked nucleotides. Each nucleotide has a sugar (deoxyribose), a nitrogenous base, plus one to three phosphate groups. Each nucleotide is linked to adjacent nucleotides in the same DNA strand by phosphodiester bonds. Phosphodiester is another sugar, made of sugar-phosphate. Nucleotides of one DNA strand link to their complementary nucleotide on the opposite DNA strand by hydrogen bonds, thus forming a pair of nucleotides, known as a base pair, or nucleotide base.

Chromosomes contain the genes, or segments of DNA, that encode for proteins of an individual. Genes contain up to

thousands of sequences of these base pairs. What distinguishes one **gene** from another is the sequence of nucleotides that code for the synthesis of a specific protein or portion of a protein. Some proteins are necessary for the structure of cells and tissues. Others, like **enzymes**, a class of active (catalyst) proteins, promote essential biochemical reactions, such as digestion, energy generation for cellular activity, or **metabolism** of toxic compounds. Some genes produce several slightly different versions of a given protein through a process of alternate **transcription** of bases pairs segments known as codons. When a chromosome is structurally faulty, or if a cell contains an abnormal number of chromosomes, the types and amounts of the proteins encoded by the genes are altered. Changes to proteins often result in serious mental and physical defects and disease.

Within the chromosomes, the DNA is tightly coiled around proteins (e.g., histones) allowing huge DNA molecules to occupy a small space within the **nucleus** of the cell. When a cell is not dividing, the chromosomes are invisible within the cell's nucleus. Just prior to cell division, the chromosomes uncoil and begin to replicate. As they uncoil, the individual chromosomes take on a distinctive appearance that allows physicians and scientists to classify the chromosomes by size and shape.

Numbers of autosomal chromosomes differ in cells of different species; but are usually the same in every cell of a given species. Sex determination cells (mature ovum and sperm) are an exception, where the number of chromosomes is halved. Chromosomes also differ in size. For instance, the smallest human chromosome, the sex chromosome Y, contains 50 million base pairs (bp), whereas the largest one, chromosome 1, contains 250 million base pairs. All 3 billion base pairs in the human genome are stored in 46 chromosomes. Human genetic information is therefore stored in 23 pairs of chromosomes (totaling 46), 23 inherited from the mother, and 23 from the father. Two of these chromosomes are sex chromosomes (chromosomes X and Y). The remaining 44 are autosomes (in 22 autosomal pairs), meaning that they are not sex chromosomes and are present in all somatic cells (i.e., any other body cell that is not a germinal cell for spermatozoa in males or an ovum in females). Sex chromosomes specify the offspring gender: normal females have two X chromosomes and normal males have one X and one Y chromosome. These chromosomes can be studied by constructing a karyotype, or organized depiction, of the chromosomes.

Each set of 23 chromosomes constitutes one allele, containing gene copies inherited from one of the progenitors. The other allele is complementary or homologous, meaning that they contain copies of the same genes and on the same positions, but originated from the other progenitor. As an example, every normal child inherits one set of copies of gene BRCA1, located on chromosome 13, from the mother and another set of BRCA1 from the father, located on the other allelic chromosome 13. Allele is a Greek-derived word that means "one of a pair," or any one of a series of genes having the same locus (position) on homologous chromosomes.

The first chromosome observations were made under light microscopes, revealing rod-shaped structures in varied sizes and conformations, commonly J- or V-shaped in eukaryotic cells and ring-shaped in bacteria. Staining reveals a pattern of light and dark bands. Today, those bands are known to correspond to regional variations in the amounts of the two nucleotide base pairs: Adenine-Thymine (A-T or T-A) in contrast with amounts of Guanine-Cytosine (G-C or C-G).

In humans, two types of cell division exist. In mitosis, cells divide to produce two identical daughter cells. Each daughter cell has exactly the same number of chromosomes. This preservation of chromosome number is accomplished through the replication of the entire set of chromosomes just prior to mitosis.

Two kinds of chromosome number defects can occur in humans: aneuploidy, an abnormal number of chromosomes, and polyploidy, more than two complete sets of chromosomes. Most alterations in chromosome number occur during meiosis. During normal meiosis, chromosomes are distributed evenly among the four daughter cells. Sometimes, however, an uneven number of chromosomes are distributed to the daughter cells.

Genetic abnormalities and diseases occur if chromosomes or portions of chromosomes are missing, duplicated or broken. Abnormalities and diseases may also occur if a specific gene is transferred from one chromosome to another (translocation), or there is a duplication or inversion of a segment of a chromosome. Down syndrome, for instance, is caused by trisomy in chromosome 21, the presence of a third copy of chromosome 21. Some structural chromosomal abnormalities have been implicated in certain cancers. For example, myelogenous leukemia is a cancer of the white blood cells. Researchers have found that the cancerous cells contain a translocation of chromosome 22, in which a broken segment switches places with the tip of chromosome 9.

In non-dividing cells, it is not possible to distinguish morphological details of individual chromosomes, because they remain elongated and entangled to each other. However, when a cell is dividing, i.e., undergoing mitosis, chromosomes become highly condensed and each individual chromosome occupies a well-defined spatial location.

Karyotype analysis was the first genetic screening utilized by geneticists to assess inherited abnormalities, like additional copies of a chromosome or a missing copy, as well as DNA content and gender of the individual. With the development of new molecular screening techniques and the growing number of identified individual genes, detection of other more subtle chromosomal **mutations** is now possible (e.g., determinations of gene mutations, levels of gene expression, etc.). Such data allow scientists to better understand disease causation and to develop new therapies and medicines for those diseases.

In mitosis, cells divide to produce two identical daughter cells. Each daughter cell has exactly the same number of chromosomes. This preservation of chromosome number is accomplished through the replication of the entire set of chromosomes just prior to mitosis.

Sex cells, such as eggs and sperm, undergo a different type of cell division called meiosis. Because sex cells each contribute half of a zygote's genetic material, sex cells must

carry only half the full **complement** of chromosomes. This reduction in the number of chromosomes within sex cells is accomplished during two rounds of cell division, called meiosis I and meiosis II. Prior to meiosis I, the chromosomes replicate and chromosome pairs are distributed to daughter cells. During meiosis II, however, these daughter cells divide without a prior replication of chromosomes. Mistakes can occur during either meiosis I and meiosis II. Chromosome pairs can be separated during meiosis I, for instance, or fail to separate during meiosis II.

Meiosis produces four daughter cells, each with half of the normal number of chromosomes. These sex cells are called haploid cells (meaning half the number). Non-sex cells in humans are called diploid (meaning double the number) since they contain the full number of normal chromosomes.

Most alterations in chromosome number occur during meiosis. When an egg or sperm that has undergone faulty meiosis and has an abnormal number of chromosomes unites with a normal egg or sperm during conception, the zygote formed will have an abnormal number of chromosomes. If the zygote survives and develops into a fetus, the chromosomal abnormality is transmitted to all of its cells. The child that is born will have symptoms related to the presence of an extra chromosome or absence of a chromosome.

See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); Enzymes; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Molecular biology and molecular genetics

CHROMOSOMES, HUMAN

• *see* CHROMOSOMES, EUKARYOTIC

CHROMOSOMES, PROKARYOTIC

The genetic material of **microorganisms**, be they prokaryotic or eukaryotic, is arranged in an organized fashion. The arrangement in both cases is referred to as a chromosome.

The **chromosomes** of prokaryotic microorganisms are different from that of eukaryotic microorganisms, such as **yeast**, in terms of the organization and arrangement of the genetic material. Prokaryotic **DNA** tends to be more closely packed together, in terms of the stretches that actually code for something, than is the DNA of eukaryotic cells. Also, the shape of the chromosome differs between many prokaryotes and **eukaryotes**. For example, the **deoxyribonucleic acid** of yeast (a eukaryotic microorganism) is arranged in a number of linear arms, which are known as chromosomes. In contrast, **bacteria** (the prototypical prokaryotic microorganism) lack chromosomes. Rather, in many bacteria the DNA is arranged in a circle.

The chromosomal material of **viruses** is can adopt different structures. Viral nucleic acid, whether DNA or **ribonucleic acid** (**RNA**) tends to adopt the circular arrangement when packaged inside the virus particle. Different types of virus can have different arrangements of the nucleic acid. However, viral DNA can behave differently inside the host, where it might remain autonomous or integrating into the host's nucleic acid. The changing behavior of the viral chromosome makes it more suitable to a separate discussion.

The circular arrangement of DNA was the first form discovered in bacteria. Indeed, for many years after this discovery the idea of any other arrangement of bacterial DNA was not seriously entertained. In bacteria, the circular bacterial chromosome consists of the double helix of DNA. Thus, the two strands of DNA are intertwined while at the same time being oriented in a circle. The circular arrangement of the DNA allows for the replication of the genetic material. Typically, the copying of both strands of DNA begins at a certain point, which is called the origin of replication. From this point, the replication of one strand of DNA proceeds in one direction, while the replication of the other strand proceeds in the opposite direction. Each newly made strand also helically coils around the template strand. The effect is to generate two new circles, each consisting of the intertwined double helix.

The circular arrangement of the so-called chromosomal DNA is mimicked by **plasmids**. Plasmids exist in the **cytoplasm** and are not part of the chromosome. The DNA of plasmids tends to be coiled extremely tightly, much more so than the chromosomal DNA. This feature of plasmid DNA is often described as supercoiling. Depending of the type of plasmid, replication may involve integration into the bacterial chromosome or can be independent. Those that replicate independently are considered to be minichromosomes.

Plasmids allow the genes they harbor to be transferred from bacterium to bacterium quickly. Often, such genes encode proteins that are involved in resistance to antibacterial agents or other compounds that are a threat to bacterial survival, or proteins that aid the bacteria in establishing an infection (such as a toxin).

The circular arrangement of bacterial DNA was first demonstrated by electron microscopy of ***Escherichia coli*** and *Bacillus subtilus* bacteria in which the DNA had been delicately released from the bacteria. The microscopic images clearly established the circular nature of the released DNA. In the aftermath of these experiments, the assumption was that the bacterial chromosome consisted of one large circle of DNA. However, since these experiments, some bacteria have been found to have a number of circular pieces of DNA, and even to have linear chromosomes and sometimes even linear plasmids. Examples of bacteria with more than one circular piece of DNA include *Brucella* species, *Deinococcus radiodurans*, *Leptospira interrogans*, *Paracoccus denitrificans*, *Rhodobacter sphaerodes*, and *Vibrio* species. Examples of bacteria with linear forms of chromosomal DNA are *Agrobacterium tumefaciens*, *Streptomyces* species, and *Borrelia* species.

The linear arrangement of the bacterial chromosome was not discovered until the late 1970s, and was not definitively proven until the advent of the technique of pulsed field gel **electrophoresis** a decade later. The first bacterium shown to possess a linear chromosome was *Borrelia burgdorferi*.

The linear chromosomes of bacteria are similar to those of eukaryotes such as yeast in that they have specialized regions of DNA at the end of each double strand of DNA. These regions are known as telomeres, and serve as boundaries to bracket the coding stretches of DNA. Telomeres also retard the double strands of DNA from uncoiling by essentially pinning the ends of each strand together with the complimentary strand.

There are two types of telomeres in bacteria. One type is called a hairpin telomere. As its name implies, the telomers bends around from the end of one DNA strand to the end of the complimentary strand. The other type of telomere is known as an invertron telomere. This type acts to allow an overlap between the ends of the complimentary DNA strands.

Replication of a linear bacterial chromosome proceeds from one end, much like the operation of a zipper. As replication moves down the double helix, two tails of the daughter double helices form behind the point of replication.

Research on bacterial chromosome structure and function has tended to focus on *Escherichia coli* as the model microorganism. This bacterium is an excellent system for such studies. However, as the diversity of bacterial life has become more apparent in beginning in the 1970s, the limitations of extrapolating the findings from the *Escherichia coli* chromosome to bacteria in general has also more apparent. Very little is known, for example, of the chromosome structure of the Archae, the primitive life forms that share features with prokaryotes and eukaryotes, and of those bacteria that can live in environments previously thought to be completely inhospitable for **bacterial growth**.

See also Genetic identification of microorganisms; Genetic regulation of prokaryotic cells; Microbial genetics; Viral genetics; Yeast genetics

CHRONIC BACTERIAL DISEASE

Chronic bacterial infections persist for prolonged periods of time (e.g., months, years) in the host. This lengthy persistence is due to a number of factors including masking of the **bacteria** from the **immune system**, invasion of host cells, and the establishment of an infection that is resistance to antibacterial agents.

Over the past three decades, a number of chromic bacterial infections have been shown to be associated with the development of the adherent, exopolysaccharide-encased populations that are termed biofilms. The constituents of the exopolysaccharide are poorly immunogenic. This means that the immune system does not readily recognize the exopolysaccharide as foreign material that must be cleared from the body. Within the blanket of polysaccharide the bacteria, which would otherwise be swiftly detected by the immune system, are protected from immune recognition. As a result, the infection that is established can persist for a long time.

An example of a chronic, **biofilm**-related **bacterial infection** is prostatitis. Prostatitis is an **inflammation** of the prostate gland that is common in men over 30 years of age. Symptoms of this disease can include intense pain, urinary complications, and sexual malfunction including infertility. Chronic bacterial prostatitis is generally associated with repeated urinary tract infections. The chronic infection is typically caused by biofilms of ***Escherichia coli***.

A second biofilm-related chronic bacterial infection is the *Pseudomonas aeruginosa* lung infection that develops early in life in some people who are afflicted with cystic fibrosis. Cystic fibrosis is due to a genetic defect that restricts the movement of salt and water in and out of cells in the lung. The resulting build-up of mucus predisposes the lungs to bacterial infection. The resulting *Pseudomonas aeruginosa* infection becomes virtually impossible to clear, due the **antibiotic resistance** of the bacteria within the biofilm. Furthermore, the body's response to the chronic infection includes inflammation. Over time, the inflammatory response is causes breathing difficulty that can be so pronounced as to be fatal.

Another chronic bacterial infection that affects the lungs is **tuberculosis**. This disease causes more deaths than any other infectious disease. Nearly two billion people are infected with the agent of tuberculosis, the bacterium *Mycobacterium tuberculosis*. As with other chronic infections, the symptoms can be mild. But, for those with a weakened immune system the disease can become more severe. Each year some three million people die of this active form of the tuberculosis infection.

Tuberculosis has re-emerged as a health problem in the United States, particularly among the poor. The development of drug resistance by the bacteria is a factor in this re-emergence.

Beginning in the mid 1970s, there has been an increasing recognition that maladies that were previously thought to be due to genetic or environmental factors in fact have their basis in chronic bacterial infections. A key discovery that prompted this shift in thinking concerning the origin of certain diseases was the demonstration by **Barry Marshall** that a bacterium called *Helicobacter pylori* is the major cause of stomach ulcers. Furthermore, there is now firm evidence of an association with chronic *Helicobacter pylori* stomach and intestinal infections and the development of certain types of intestinal cancers.

At about the same time the bacterium called *Borrelia burgdorferi* was established to be the cause of a debilitating disease known as **Lyme disease**. The spirochaete is able to establish a chronic infection in a host. The infection and the host's response to the infection, causes arthritis and long-lasting lethargy.

As a final recent example, **Joseph Penninger** has shown that the bacterium *Chlamydia trachomatis* is the agent that causes a common form of heart disease. The bacterium chronically infects a host and produces a protein that is very similar in three-dimensional structure to a protein that composed a heart valve. The host's immune response to the bacterial protein results in the deterioration of the heart protein, leading to heart damage.

Evidence is accumulating that implicates chronic bacterial infection with other human ailments including schizo-

phrenia and Alzheimer's disease. While not yet conclusive, the involvement of chronic bacterial infections in maladies that have hitherto not been suspected of having a bacterial origin will not be surprising.

Research efforts to prevent chronic bacterial infections are focusing on the prevention of the surface adhesion that is a hallmark of many such infections. Molecules that can competitively block the sites to which the disease-causing bacteria bind have shown promising results in preventing infections in the laboratory setting.

See also Bacteria and bacterial infection; Biofilm formation and dynamic behavior; Immunity, active, passive and delayed

CJD DISEASE • *see* BSE AND CJD DISEASE

CLINICAL MICROBIOLOGY • *see* MICROBIOLOGY, CLINICAL

CLINICAL TRIALS, TYPES • *see* MICROBIOLOGY, CLINICAL

CLONING: APPLICATIONS TO BIOLOGICAL PROBLEMS

Human proteins are often used in the medical treatment of various human diseases. The most common way to produce proteins is through human cell **culture**, an expensive approach that rarely results in adequate quantities of the desired protein. Larger amounts of protein can be produced using **bacteria** or **yeast**. However, proteins produced in this way lack important post-translational modification steps necessary for protein maturation and proper functioning. Additionally, there are difficulties associated with the purification processes of proteins derived from bacteria and yeast. Scientists can obtain proteins purified from blood but there is always risk of **contamination**. For these reasons, new ways of obtaining low-cost, high-yield, purified proteins are in demand.

One solution is to use transgenic animals that are genetically engineered to express human proteins. **Gene** targeting using nuclear transfer is a process that involves removing nuclei from cultured adult cells engineered to have human genes and inserting the nuclei into egg cells void of its original **nucleus**.

Transgenic cows, sheep, and goats can produce human proteins in their milk and these proteins undergo the appropriate post-translational modification steps necessary for therapeutic efficacy. The desired protein can be produced up to 40 grams per liter of milk at a relatively low expense. Cattle and other animals are being used experimentally to express specific genes, a process known as "pharming." Using cloned transgenic animals facilitates the large-scale introduction of foreign genes into animals. Transgenic animals are cloned using nuclear gene transfer, which reduces the amount of experimental animals used as well as allows for specification of the sex of the progeny resulting in faster generation of breeding stocks.

Medical benefits from cloned transgenic animals expressing human proteins in their milk are numerous. For example, human serum albumin is a protein used to treat patients suffering from acute burns and over 600 tons are used each year. By removing the gene that expresses bovine serum albumin, cattle clones can be made to express human serum albumin. Another example is found at one biotech company that uses goats to produce human tissue plasminogen activator, a human protein involved in blood clotting cascades. Another biotech company has a flock that produces alpha-1-antitrypsin, a drug currently in clinical trials for the use in treating patients with cystic fibrosis. Cows can also be genetically manipulated using nuclear gene transfer to produce milk that does not have lactose for lactose-intolerant people. There are also certain proteins in milk that cause immunological reactions in certain individuals that can be removed and replaced with other important proteins.

There is currently a significant shortage of organs for patients needing transplants. Long waiting lists lead to prolonged suffering and people often die before they find the necessary matches for transplantation. Transplantation technology in terms of hearts and kidneys is commonplace, but very expensive. Xenotransplantation, or the transplantation of organs from animals into humans, is being investigated, yet graft versus host rejection remains problematic. As an alternative to xenotransplantation, stem cells can be used therapeutically, such as in blood disorders where blood stem cells are used to deliver normal blood cell types. However, the availability of adequate amount of stem cells is a limiting factor for stem cell therapy.

One solution to supersede problems associated with transplantation or stem cell therapy is to use cloning technology along with factors that induce differentiation. The process is termed, "therapeutic cloning" and might be used routinely in the near future. It entails obtaining adult cells, reprogramming them to become stem cell-like using nuclear transfer, and inducing them to proliferate but not to differentiate. Then factors that induce these proliferated cells to differentiate will be used to produce specialized cell types. These now differentiated cell types or organs can then be transplanted into the same donor that supplied the original cells for nuclear transfer.

Although many applications of cloning technology remain in developmental stages, the therapeutic value has great potential. With technological advancements that allow scientists to broaden the applications of cloning becoming available almost daily, modern medicine stands to make rapid improvements in previously difficult areas.

See also DNA hybridization; Immunogenetics; Microbial genetics; Transplantation genetics and immunology

CLOSTRIDIUM • *see* BOTULISM

COAGULASE

Coagulase is an enzyme that is produced by some types of **bacteria**. The enzyme clots the plasma component of the blood. The only significant disease-causing bacteria of humans that produces coagulase is *Staphylococcus aureus*.

In the human host, the action of coagulase produces clotting of the plasma in the immediate vicinity of the bacterium. The resulting increased effective diameter of the bacterium makes it difficult for the defense reactions of the host to deal with the infecting cell. In particular, the defensive mechanism of **phagocytosis**, where the bacterium is engulfed by a host cell and then dissolved, is rendered ineffective. This enables the bacterium to persist in the presence of a host immune response, which can lead to the establishment of n infection. Thus, coagulase can be described as a disease-causing (or virulence) factor of *Staphylococcus aureus*

A test for the presence of active coagulase distinguishes the aureus Staphylococcus from the non-aureus **Staphylococci**. *Staphylococcus aureus* is one of the major causes of hospital-acquired infection. **Antibiotic resistance** of this strain is a major concern. In the non-aureus, coagulase-negative group, *Staphylococcus epidermidis* is a particular concern. This strain is also an important disease-causing organism in hospital settings and can establish infections on artificial devices inserted into the body. The ability to quickly and simply differentiate the two different types of *Staphylococcus* from each other enables the proper treatment to be started before the infections become worse.

In the test, the sample is added to rabbit plasma and held at 37° C or a specified period of time, usually bout 12 hours. A positive test is the formation of a visible clump, which is the clotted plasma. Samples must be observed for clotting within 24 hours. This is because some strains that produce coagulase also produce an enzyme called fibrinolysin, which can dissolve the clot. Therefore, the absence of a clot after 24 hours is no guarantee that a clot never formed. The formation of a clot by 12 hours and the subsequent disappearance of the clot by 24 hours could produce a so-called false negative if the test were only observed at the 24-hour time.

See also Biochemical analysis techniques; Laboratory techniques in microbiology

COHEN, STANLEY N. (1935-)

American geneticist

Modern biology, **biochemistry**, and genetics were fundamentally changed in 1973 when Stanley N. Cohen, **Herbert W. Boyer**, Annie C. Y. Chang, and Robert B. Helling developed a technique for transferring **DNA**, the molecular basis of heredity, between unrelated species. Not only was DNA propagation made possible among different bacterial species, but successful **gene** insertion from animal cells into bacterial cells was also accomplished. Their discovery, called recombinant DNA or genetic engineering, introduced the world to the age of modern **biotechnology**.

As with any revolutionary discovery, the benefits of this new technology were both immediate and projected. Immediate gains were made in the advancement of fundamental biology by increasing scientists' knowledge of gene structure and function. This knowledge promised new ways to overcome disease, increase food production, and preserve renewable resources. For example, the use of recombinant DNA methodology to overcome **antibiotic resistance** on the part of **bacteria** anticipated the development of better vaccines. A new source for producing insulin and other life-sustaining drugs had the potential to be realized. And, by creating new, nitrogen-fixing organisms, it was thought that food production could be increased, and the use of expensive, environmentally harmful nitrogen fertilizers eliminated. Genetic engineering also offered the promise of nonpolluting energy sources, such as hydrogen-producing algae. In the decades following the discovery of the means for propagating DNA, many assumptions regarding the benefits of genetic engineering have proved to be viable, and the inventions and technology that were by-products of genetic engineering research became marketable commodities, propelling biotechnology into a dynamic new industry.

Stanley N. Cohen was born in Perth Amboy, New Jersey, to Bernard and Ida Stolz Cohen. He received his undergraduate education at Rutgers University, and his M.D. degree from the University of Pennsylvania in 1960. Then followed medical positions at Mt. Sinai Hospital in New York City, University Hospital in Ann Arbor, Michigan, the National Institute for Arthritis and Metabolic Diseases in Bethesda, Maryland, and Duke University Hospital in Durham, North Carolina. Cohen completed postdoctoral research in 1967 at the Albert Einstein College of Medicine in the Bronx, New York. He joined the faculty at Stanford University in 1968, was appointed professor of medicine in 1975, professor of genetics in 1977, and became Kwoh-Ting Li professor of genetics in 1993.

At Stanford Cohen began the study of **plasmids**—bits of DNA that exist apart from the genetic information-carrying **chromosomes**—to determine the structure and function of plasmid genes. Unlike species ordinarily do not exchange genetic information. But Cohen found that the independent **plasmids** had the ability to transfer DNA to a related-species cell, though the phenomenon was not a commonplace occurrence. In 1973 Cohen and his colleagues successfully achieved a DNA transfer between two different sources. These functional molecules were made by joining two different plasmid segments taken from ***Escherichia coli***, a bacteria found in the colon, and inserting the combined plasmid DNA back into ***E. coli*** cells. They found that the DNA would replicate itself and express the genetic information contained in each original plasmid segment. Next, the group tried this experiment with an unrelated bacteria, *Staphylococcus*. This, too, showed that the original *Staphylococcus* plasmid genes would transfer their biological properties into the *E. coli* host. With this experiment, the DNA barrier between species was broken. The second attempt at DNA replication between unlike species was that of animal to bacteria. This was successfully undertaken with the insertion into *E. coli* of genes

taken from a frog. This experiment had great significance for human application; bacteria containing human genetic information could now be used to create the body's own means for fighting disease and birth disorders. The biological **cloning** methods used by Cohen and other scientists came to be popularly known as genetic engineering. The cloning process consisted of four steps: separating and joining DNA molecules acquired from unlike species; using a gene carrier that could replicate itself, as well as the unlike DNA segment joined to it; introducing the combined DNA molecule into another bacterial host; and selecting out the clone that carries the combined DNA.

DNA research not only added to the store of scientific knowledge about how genes function, but also had practical applications for medicine, agriculture, and industry. By 1974, there was already speculation in the media about the benefits that could accrue from gene transplant techniques. The creation of bacteria "factories" that could turn out large amounts of life-saving medicines was just one possibility. In fact, insulin made from bacteria was just seven years from becoming a reality. Still in the future at that time, but proved possible within two decades, were supermarket tomatoes hardy enough to survive cross-country trucking that taste as good as those grown in one's own garden. Using DNA technology, other plants were also bred for disease and pollution resistance. Scientists also projected that nitrogen-fixing microbes, such as those that appear in the soil near the roots of soybeans and other protein-rich plants, could be duplicated and introduced into corn and wheat fields to reduce the need for petroleum-based nitrogen fertilizer. Cohen himself said, in an article written for the July 1975 issue of *Scientific American:* "Gene manipulation opens the prospect of constructing bacterial cells, which can be grown easily and inexpensively, that will synthesize a variety of biologically produced substances such as **antibiotics** and hormones, or **enzymes** that can convert sunlight directly into food substances or usable energy."

When news of this remarkable research became widespread throughout the general population during the 1970s and 1980s, questions were raised about the dangers that might be inherent in genetic engineering technology. Some people were concerned that the potential existed for organisms altered by recombinant DNA to become hazardous and uncontrollable. Although safety guidelines had long been in place to protect both scientists and the public from disease-causing bacteria, toxic chemicals, and radioactive substances, genetic engineering seemed, to those outside the laboratory, to require measures much more restrictive. Even though, as responsible scientists, Cohen and others who were directly involved with DNA research had already placed limitations on the types of DNA experiments that could be performed, the National Academy of Sciences established a group to study these concerns and decide what restrictions should be imposed. In 1975, an international conference was held on this complicated issue, which was attended by scientists, lawyers, legislators, and journalists from seventeen countries. Throughout this period, Cohen spent much time speaking to the public and testifying to government agencies regarding DNA technology, attempting to ease concerns regarding DNA experimentation.

Cohen contended that public outcry over the safety of DNA experiments resulted in an overly cautious approach that slowed the progress of DNA research and reinforced the public's belief that real, not conjectural, hazards existed in the field of biotechnology. In an article on this subject published in 1977 for *Science* he pointed out that during the initial recombinant DNA experiments, billions of bacteria played host to DNA molecules from many sources; these DNA molecules were grown and propagated "without hazardous consequences so far as I am aware. And the majority of these experiments were carried out prior to the strict containment procedures specified in the current federal guidelines."

The controversy over the safety of DNA technology absorbed much of Cohen's time and threatened to obscure the importance of other plasmid research with which he was involved during those years. For instance, his work with bacterial **transposons**, the "jumping genes" that carry antibiotic resistance, has yielded valuable information about how this process functions. He also developed a method of using "reporter genes" to study the behavior of genes in bacteria and eukaryotic cells. In addition, he has searched for the mechanism that triggers plasmid inheritance and **evolution**. Increased knowledge in this area offers the medical community more effective tools for fighting antibiotic resistance and better understanding of genetic controls.

Cohen has made the study of plasmid biology his life's work. An introspective, modest man, he is most at home in the laboratory and the classroom. He has been at Stanford University for more than twenty-five years, serving as chair of the Department of Genetics from 1978 to 1986. He is the author of more than two hundred papers, and has received many awards for his scientific contributions, among them the Albert Lasker Basic Medical Research Award in 1980, the Wolf Prize in Medicine in 1981, both the National Medal of Science and the LVMH Prize of the Institut de la Vie in 1988, the National Medal of Technology in 1989, the American Chemical Society Award in 1992, and the Helmut Horten Research Award in 1993. Cohen has held memberships in numerous professional societies, including the National Academy of Sciences (chairing the genetics section from 1988 to 1991), the Institute of Medicine of the National Academy, and the Genetics Society of America. In addition, he served on the board of the *Journal of Bacteriology* in the 1970s, and was associate editor of *Plasmid* from 1977 to 1986. Since 1977, he has been a member of the Committee on Genetic Experimentation for the International Council of Scientific Unions. Married in 1961 to Joanna Lucy Wolter, and the father of two children, Cohen lives mostly near Stanford University in a small, rural community. Free time away from his laboratory and his students has been spent skiing, playing five-string banjo, and sailing his aptly named boat, *Genesis*.

See also Microbial genetics

COHN, FERDINAND JULIUS (1828-1898)

German microbiologist

Ferdinand Cohn, a founder of modern microbiology, became the first to recognize and study bacteriology as a separate science. Cohn developed a system for classifying **bacteria** and discovered the importance of heat-resistant endospores. Additionally, Cohn recognized that both pathogens and nonpathogens could be found in drinking water and spoke of the importance of analyzing drinking water. Finally, Cohn worked with **Robert Koch** on the development of the etiology of the **anthrax** bacillus.

Cohn initially began his studies in botany at the University of Breslau in 1844. After being denied entry into the doctoral program in 1846 because of his Jewish heritage, Cohn moved to Berlin. There he completed his doctoral degree in 1847, at the age of 19, on the structure and germination of seeds.

After returning to Breslau in 1849, Cohn was presented with a top of the line **microscope** from his father. There he studied the cell biology of plants including the growth and division of plant cells, plasma streaming, cell differentiation, and cellular structures. In time, Cohn's studies were redirected toward algae, **protozoa**, **fungi**, and bacteria. His efforts on the developmental and sexual cycles of these **microorganisms** led to important advancements in cell biology.

At that time, bacteriology was an emerging field and although scientists knew that bacteria existed, they had failed to isolate bacteria in pure cultures. Scientists began to name bacteria without regard for someone else that had already observed and named the very same bacteria. Moreover, scientists believed bacteria to be a single species and that variations observed were due to different stages of development. Cohn recognized that bacteria could not be classified as a single species and developed a system for classifying them. He proposed that bacteria could be divided into groups based on whether they had similar development, chemical make-up, or descent. In 1875, he defined bacteria as "chlorophyll-less cells of characteristic shape that multiply by cross division and live as singe cells, filamentous cell chains, or cell aggregates." Eventually he extended his definition to include that "bacteria can be divided into distinct species with typical characteristics, which are transmitted to the following generations when bacteria multiply and that variations exist within each species."

After comprehensive studies of bacteria, Cohn believed that bacteria were related to algae and should thus be classified in the plant kingdom. Additionally, Cohn studied the growth of bacteria and found that in some bacteria organic substances were broken down in the presence of nitrogen. He also claimed that carbon dioxide could not be utilized as a carbon source in bacteria. It was not until 1890 when Sergei N. Winogradsky disproved this statement and discovered autotrophy.

Cohn's initial classification of bacteria consisted of four groups based on shape: Sphaerobacteria (sphere-shaped), Microbacteria (rod-shaped), Desmobacteria (filamentous), and Spirobacteria (screw-like shaped). Of those four groups the genus *Micrococcus* was classified as Sphaerobacteria, *Bacterium* was classified as Microbacteria, *Bacillus* and *Vibrio* were classified as Desmobacteria, and *Spirillum* and *Spirochaeta* were classified as Spirobacteria. Some of the genera could be further divided into subcategories.

Through the studies of *Bacillus subtilis* Cohn was able to disprove the earlier theory of spontaneous generation. Cohn recognized that some solutions were easily sterilized by heat, requiring only a few minutes of boiling, while other solutions required several hours of boiling. He found that still others, such as hay infusions, could not be sterilized at all. Cohn discovered heat-resistant structures called endospores, not spontaneous generation, were responsible for tainting sterilized cultures. Endospores are not killed in boiling water while the vegetative cells are. It was the heat resistant endospores from which bacteria grew, discounting the old theory of spontaneous generation.

Early on Cohn assisted in diagnosing fungal infections of crops and provided treatment options to the farmers for these plant diseases. Additionally, Cohn recognized that water sources were capable of harboring and transferring infectious diseases to humans. It was Robert Koch who first identified the pathogen that caused cholera in the drinking water; however, Cohn also analyzed the drinking water and found disease and non-disease causing microorganisms. Cohn developed a system for chemical analysis of water and claimed that drinking water should be monitored for microorganisms on a regular basis.

Later when Robert Koch was studying anthrax bacillus, Koch sought the help of Cohn. Cohn realized the importance of studying the disease causing anthrax bacillus and worked with Koch to further investigate the etiology of the bacteria. In 1875, Cohn founded the journal *Beitrage zur Biologie der Pflanzen* and published Koch's findings on anthrax bacillus in 1877.

See also Water quality; Cell cycle and cell division; History of microbiology

COLD, COMMON

Dedicated researchers have searched for a cure or even an effective treatment for the common cold (rhinitis) for years. Discovering or constructing the agent that will be universally lethal to all the cold-causing **viruses** has been fruitless. A drug that will kill only one or two of the viruses would be of little use since the patient would not know which of the viruses was the one that brought on his cold.

The common cold differs in several ways from **influenza** or the flu. Cold symptoms develop gradually and are relatively mild. The flu has a sudden onset and has more serious symptoms the usually put the sufferer to bed, and the flu lasts about twice as long as the cold. Also influenza can be fatal, especially to elderly persons, though the number of influenza viruses is more limited than the number of cold viruses, and vaccines are available against certain types of flu.

Rhinoviruses, **adenoviruses**, influenza viruses, parainfluenza viruses, syncytial viruses, echoviruses, and coxsackie viruses—all have been implicated as the agents that cause the

Sneezing is a symptom of the common cold.

runny nose, cough, sore throat, and sneezing that advertise that you have a cold. More than 200 viruses, each with its own favored method of being passed from one person to another, its own gestation period, each different from the others, wait patiently to invade the mucous membranes that line the nose of the next cold victim.

Passing the cold-causing virus from one person to the next can be done by sneezing onto the person, by shaking hands, or by an object handled by the infected person and picked up by the next victim. Oddly, direct contact with the infected person, as in kissing, is not an efficient way for the virus to spread. Only in about 10% of such contacts does the uninfected person get the virus. Walking around in a cold rain will not cause a cold. Viruses like warm, moist surroundings, so they thrive indoors in winter. Colds are easily passed in the winter, because people spend more time indoors then than they do outdoors. However, being outdoors in cold weather can dehydrate the mucous membranes in the nose and make them more susceptible to infection by a rhinovirus.

In addition, cold-causing viruses mutate with regularity. Each time it is passed from one person to the next, the virus changes slightly, so it is not the virus the first person had. Viruses are obligate **parasites**, meaning that they can carry out their functions only when they invade another living cell.

The virus has a tough envelope surrounding its nucleic acids, the genetic material for any living thing. Once it invades the body, the virus waits to be placed in the location in which it can function best. Once there, it attaches to a cell by means of receptor areas on its envelope and on the cell membrane. The viral nucleic acid then is inserted into the cell **nucleus** and it takes over the functions of the nucleus, telling it to reproduce viruses.

Taking regular doses of vitamin C will not ward off a cold. However, high doses of vitamin C once a person has a cold may help to alleviate symptoms and reduce discomfort. Over-the-counter drugs to treat colds treat only the symptoms. They may dry up the patient's runny nose, but after a few days the nose will compensate and overcome the effects of the medication and begin to drip again. The runny nose is from the loss of plasma from the blood vessels in the nose. Some researchers assert the nose drip is a defensive mechanism to prevent the invasion of other viruses. **Antibiotics** such as **penicillin** are useless against the cold because they do not affect viruses.

Scientists agree that the old wives' remedy of chicken soup can help the cold victim, but so can any other hot liquid. The steam and heat produced by soup or tea helps to liquefy the mucus in the sinus cavities, allowing them to drain, reducing the pressure and making the patient feel better. The remedy is temporary and has no effect on the virus. Colds are usually self-limiting, and recovery usually occurs within a week.

See also Cold, viruses; Infection and resistance; Viruses and responses to viral infection

COLD, VIRUSES

The cold is one of the most common illnesses of humans. In the Unites States alone, there are more than one billion colds each year. Typically a cold produces sneezing, scratchy throat, and a runny nose for one or two weeks. The causes of the common **cold** are **viruses.**

More than 200 different viruses can cause a cold. Rhinoviruses account for anywhere from 35% to over half of all colds, particularly in younger and older people. This has likely been the case for millennia. Indeed, the name Rhinovirus is from the Greek word *rhin*, meaning, "nose." There are over one hundred different types of Rhinovirus, based on the different proteins that are on the surface of the virus particle. Rhinovirus belongs to the virus family Picornaviridae. The genetic material of the virus is **ribonucleic acid** (**RNA**) and the genome is of a very small size.

Rhinovirus is spread from one person to another by "hand to hand" contact, that is, by physical contact or from one person sneezing close by another person. The virus needs to inside the human body to be able to replicate. The internal temperature of the body, which is normally between 97–99°F (36.1–37.2° C) is perfect for Rhinovirus. If the temperature varies only a few degrees either way of the window, the virus will not replicate.

Rhinovirus has been successful in causing colds for such as long time because of the large number of antigenic types of the virus that exist. Producing a **vaccine** against the virus would require the inclusion of hundreds of antibodies to the hundreds of different possible antigens. This is not practical to achieve. Furthermore, not all the Rhinovirus antigens that are important in generating a cold are exposed at the surface. So, even if a corresponding **antibody** were present, neutralization of the **antigen** via the binding of the antibody with the antigen would not occur. Another factor against vaccine development is the difficulty in being able to grow Rhinovirus in the laboratory.

Another virus that causes colds are members of the Coronavirus family. The name of the virus derives from the distinctive flexible shape and appearance of the virus particle. Surface projections give the virus a crown-like, or corona, appearance. There are more than 30 known strains of Coronavirus. Of these, three or four from the genus *Coronavirus* can infect humans. Cattle, pigs, rodents, cats, dogs, and birds are also hosts. Members of the genus *Torovirus* can also cause **gastroenteritis**.

Coronavirus has been known since 1937, when it was isolated from chickens. It was suspected of being a cause of colds, but this could not be proven until the 1960s, when techniques to grow the virus in laboratory cultures were devised. Like Rhinovirus, Coronavirus also contains RNA. However, in contrast to the same amount of genetic material carried in Rhinoviruses, the genome of the Coronavirus is the largest of all the RNA-containing viruses.

Other viruses account for 10–15% of colds in adults. These **adenoviruses**, coxsackieviruses, echoviruses, orthomyxoviruses (including the **influenza** A and B viruses), paramyxoviruses, respiratory syncytial virus and enteroviruses can also cause other, more severe illnesses.

Aside from vaccines, various "home remedies" to the common cold exist. Larger than normal doses of Vitamin C have been claimed to lessen the symptoms or prevent the common cold. The evidence for this claim is still not definitive. Another remedy, mythologized as an example of a mother's care for her children, is chicken soup. Studies have demonstrated that chicken soup may indeed shorten the length of a cold and relieve some of the symptoms. The active ingredient(s), if any, that are responsible are not known, however. For now, the best treatment for a cold is to attempt to relieve the symptoms via such home remedies and over the counter medications. Nasal decongestants decrease the secretions from the nose and help relieve congestion. Antihistamines act to depress the **histamine** allergic response of the **immune system**. This has been claimed to help relieve cold symptoms. Analgesics relieve some of pain and fever associated with a cold.

Some so-called alternative medications may have some benefit. For example, lozenges composed of zinc can sometimes reduce the duration of the common cold, perhaps due to the need for zinc by the immune system. Echinacea is known to stimulate white blood cell activity.

See also Virology

COLIFORM BACTERIA • *see* ESCHERICHIA COLI (E. COLI)

COLONY AND COLONY FORMATION

A colony is population of a single type of microorganism that is growing on a solid or semi-solid surface. **Bacteria**, **yeast**, **fungi**, and molds are capable of forming colonies. Indeed, when a surface is available, these microbes prefer the colonial mode of growth rather than remaining in solution.

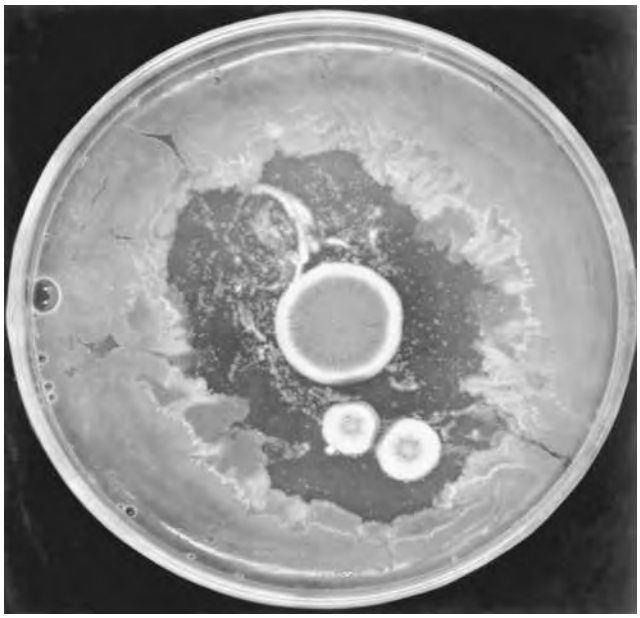
On a colonized solid surface, such as the various growth media used to **culture microorganisms**, each colony arises from a single microorganism. The cell that initially adheres to the surface divides to form a daughter cell. Both cells subsequently undergo another round of growth and division. This cycle is continually repeated. After sufficient time, the result is millions of cells piled up in close association with each other. This pile, now large enough to be easily visible to the unaided eye, represents a colony.

The appearance of a colony is governed by the characteristic of the organism that is the building block of that colony. For example, if a bacterium produces a color (the organism is described as being pigmented), then the colony can appear colored. Colonies can be smooth and glistening, rough and dry looking, have a smooth border or a border that resembles an undulating coastline, and can have filamentous appearance extensions sticking up into the air above the colony.

The visual appearance of a colony belies the biochemical complexities of the population within. For example, in a bacterial colony, the organisms buried in the colony and those near the more aged center of the colony are not as robustly growing as those bacteria at the periphery of the colony. Indeed, researchers have shown that the various phases of growth found when bacteria grow in a liquid growth medium in a flask (fast-growing and slower-growing bacteria, dying bacteria, and newly forming bacteria) all occur simultaneously in various regions of a colony. Put another way, within colonies, cells will have different phenotypes (structure) and genotypes (expression of genes).

Variations of **phenotype** and **genotype** have been elegantly demonstrated using a variant of ***Escherichia coli*** that differentially expresses a **gene** for the **metabolism** of a sugar called lactose depending on the growth rate of the bacteria. Growth on a specialized medium produces a blue color in those cells were the gene is active. Colonies of the variant will have blue-colored sectors and colorless sectors, corresponding to populations of bacteria that are either expressing the lactose-metabolizing gene or where the gene is silent.

The nature of the solid surface also affects the formation of a colony. For example, nutrients can diffuse deeper into a semi-solid growth medium than in a very stiff medium. Colonies of *Bacillus subtilis* bacteria tend to form more wavy, fern-like edges to their colonies in the semi-solid medium. This is because of uneven distribution of nutrients. Those bacteria in a relatively nutrient-rich zone will be able to grow



Colonies of *Penicillium notatus*, showing surrounding zone of bacterial inhibition. This is the phenomenon noted by Fleming in 1929, that led to his discovery of Penicillin. Undated.

faster, and often grow in the direction of the nutrient source. Even the shape of the bacteria changes from an oval to a longer form in these fast growing regions. The molecular basis of this shape **transformation** remains unresolved.

In another example of the influence of the surface on colony dynamics, the periphery of colonies grown on wet surfaces contains very motile (moveable) bacteria. Their motion is constrained by the high number of bacteria. The results is the formation of so-called "whirls and jets" that form, disappear and re-form. These motions, which appear under the light **microscope** to be very random and chaotic, are in fact very highly organized and helps drive the further formation of the colony.

Another phenomenon of colony formation is the communication between constituent cells. This is also known as "cross-talk." Cells of the amoeba *Dictyostelium discoideum*, for example, can actually signal one another when growing in a colony, especially in nutrient-poor environments. Cells that encounter nutrients emit a compound called cyclic adenosine monophosphate (cAMP). The subsequent growth of cells is in the direction of the increasing cAMP concentration. Visually, a spiraling pattern of growth results. Mounds of amoebas also form. The microbes at the top of the mounds produce spores that can become dispersed by air movement, allowing the colonization and new colony formation of other surfaces.

Chemical signalling within a colony has also been demonstrated in yeast, such as *Candida mogii* and in bacteria, such as *Escherichia coli*.

See also Agar and agarose; Biofilm formation and dynamic behavior

COLWELL, RITA R. (1934-)

American marine microbiologist

Rita R. Colwell is a leader in marine **biotechnology**, the application of molecular techniques to marine biology for the harvesting of medical, industrial and aquaculture products from the sea. As a scientist and professor, Colwell has investigated the ecology, physiology, and evolutionary relationships of marine **bacteria**. As a founder and president of the University of Maryland Biotechnology Institute, she has nurtured a vision to improve the environment and human health by linking **molecular biology** and genetics to basic knowledge scientists have gleaned from life and chemistry in the oceans.

Rita Rossi was born in Beverly, Massachusetts, the seventh of eight children to parents Louis and Louise Di Palma Rossi. Her father was an Italian immigrant who established his own construction company, and her mother was an artistic woman who worked to help ensure her children would have a good education. She died when her daughter was just thirteen years old, but she had expressed pride in her success in school. In the sixth grade, after Rossi had scored higher on an IQ test than anyone previously in her school, the principal sternly stressed that Rossi had the responsibility to go to college. Eventually, Rossi received a full scholarship from Purdue University. She earned her B.S. degree with distinction in bacteriology in 1956. Although she had been accepted to medical school, Rossi chose instead to earn a master's degree so that she could remain at the same institution as graduate student Jack Colwell, whom she married on May 31, 1956. Colwell would have continued her studies in bacteriology, but the department chairman at Purdue informed her that giving fellowship money to women would have been a waste. She instead earned her master's degree in the department of genetics. The University of Washington, Seattle, granted her a Ph.D. in 1961 for work on bacteria commensal to marine animals, which is the practice of an organism obtaining food or other benefits from another without either harming or helping it. Colwell's contributions included establishing the basis for the systematics of marine bacteria.

In 1964, Georgetown University hired Colwell as an assistant professor, and gave her tenure in 1966. Colwell and her research team were the first to recognize that the bacterium that caused cholera occurred naturally in estuaries. They isolated the bacterium from Chesapeake Bay and in ensuing years sought to explain how outbreaks in human populations might be tied to the seasonal abundance of the host organisms in the sea, particularly **plankton**. In 1972, Colwell took a tenured professorship at the University of Maryland. Her studies expanded to include investigations on the impact of marine pollution at the microbial level. Among her findings was that the presence of oil in estuarine and open ocean water was associated with the numbers of bacteria able to break down oil. She studied whether some types of bacteria might be used to treat oil spills. Colwell and her colleagues also made a discovery that held promise for improving oyster yields in aquaculture—a bacterial film

formed on surfaces under water that attracted oyster larvae to settle and grow.

In the spirit of using knowledge gained from the sea to benefit humans and the environment, Colwell prepared a seminal paper on marine biotechnology published in the journal *Science* in 1983. It brought attention to the rich resources of the ocean that might be tapped for food, disease-curing drugs, and environmental clean-up by the applications of genetic engineering and **cloning**. In order to realize the potential of marine biotechnology as originally outlined in her 1983 paper, Colwell helped foster the concept and growth of the University of Maryland Biotechnology Institute, established in 1987. As president of the U.M.B.I., she has formed alliances between researchers and industry and has succeeded in raising funds to develop the center as a prestigious biotech research complex.

In addition, Colwell has held numerous professional and academic leadership positions throughout her career and is a widely published researcher. At the University of Maryland, Colwell was director of the Sea Grant College from 1977 to 1983. She served as president of Sigma Xi, the American Society for Microbiology, and the International Congress of Systematic and Evolutionary Biology, and was president-elect of the American Association for the Advancement of Science. Colwell has written and edited more than sixteen books and over four hundred papers and articles. She also produced an award-winning film, *Invisible Seas*. Her honors included the 1985 Fisher Award of the American Society for Microbiology, the 1990 Gold Medal Award of the International Institute of Biotechnology, and the 1993 Phi Kappa Phi National Scholar Award.

Colwell is the mother of two daughters who pursued careers in science. She is an advocate for equal rights for women, and one of her long-standing aspirations is to write a novel about a woman scientist. Her hobbies include jogging and competitive sailing.

See also Bioremediation; *E. coli* O157:H7 infection; Economic uses and benefits of microorganisms; Water purification

COMBINED IMMUNODEFICIENCY • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

COMMERCIAL USES OF MICROORGANISMS • *see* ECONOMIC USES AND BENEFITS OF MICROORGANISMS

COMMON VARIABLE IMMUNODEFICIENCY DISEASE (CVID) • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

COMPETITIVE EXCLUSION OF BACTERIAL ADHESION • *see* ANTI-ADHESION METHODS

COMPLEMENT

Complement refers to a series of some 30 proteins that enhance the bacterial killing effect of antibodies. This complementation involves facilitating the engulfing of **bacteria** by immune cells in the process known as **phagocytosis**, or by the puncturing of the bacterial membrane. Additionally, complement helps dispose of antigen-antibody complexes that form in the body.

The various complement proteins circulate throughout the bloodstream in an inactive form. When one of the proteins is converted to an active form upon interaction with an antigen-antibody complex, a series of reactions is triggered. The activation step involves the cleaving, or precise cutting, of the particular complement protein. The cleavage turns the complement protein into a protease, a protein that is itself capable of cleaving other proteins. In turn, cleavage of a second complement protein makes that protein a protease. The resulting cleavage reaction generates a series of active complement proteins. These reactions, known as the complement cascade, occur in an orderly sequence and are under precise regulation.

The reactions involve two pathways. One is known as the classical complement activation pathway. The end result is an enzyme that can degrade a protein called C3. The other pathway is known as the alternative pathway. The second pathway does not require the presence of **antibody** for the activation of complement. Both pathways result in the formation of an entity that is called the membrane attack complex. The complex is actually a channel that forms in the bacterial membrane. Under the magnification of the **electron microscope**, a bacterial membrane that is a target of the complement system appears riddled with holes.

The channels that form in a membrane allow the free entry and exit of fluids and molecules. Because the concentration of various ions is higher inside the bacterium than outside, fluid will flow inward to attempt to balance the concentrations. As a result, the bacterium swells and bursts.

Other reaction products of the complement cascade trigger an inflammatory immune response. In addition, the invading bacteria are coated with an immune molecule (C3b) that makes the bacteria more recognizable to phagocytes. This process is called **opsonization**. The phagocytes then engulf the bacteria and degrade them.

Tight control over the activity of the complement system is essential. At least 12 proteins are involved in the regulation of complement activation. Defects in this control, or the operation of the pathways, result in frequent bacterial infections.

See also Immune system; Infection and control

COMPLEMENT DEFICIENCY • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

COMPLETED TESTS • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

COMPOSTABLES ONLY
NO MORNING GLORY
COMFREY ROOTS
QUACK GRASS
PLACE THESE THINGS IN THE ADJACENT SHORT BINS
PLEASE NO PLASTIC OR STRING IN THESE BINS

A compost bin.

COMPLEX MEDIA • *see* GROWTH AND GROWTH MEDIA

COMPOSTING, MICROBIOLOGICAL ASPECTS

Composting is the conversion of organic material, such as plant material and household foodstuffs, to a material having a soil-like consistency. This material is called compost. The composting process, which is one of decomposition, relies upon living organisms. Insects and earthworms participate. **Bacteria** and **fungi** are of fundamental importance.

Composting is a natural process and enables nutrients to be cycled back into an ecosystem. The end products of composition are compost, carbon dioxide, water and heat.

The decomposition process is achieved mainly by bacteria and fungi. Bacteria predominate, making up 80 to 90% of the **microorganisms** found in compost.

There are several phases to the composting process, which involve different microorganisms. The first phase, which lasts a few days after addition of the raw material to the compost pile, is a moderate temperature (mesophilic) phase. As microbial activity produces decomposition and by-products, including heat, a high-temperature (thermophilic) phase takes over. The dominant microorganisms will become those that are adapted to life at higher temperature, the so-called thermophiles. The high-temperature (thermophilic) phase will last anywhere from a few days to a few months. Finally, as decomposition activity of the microbial population slows and ceases, a cooling-down phase ensues over several months.

Initially, the mesophilic microorganisms break down compounds that readily dissolve in water. This decomposition is rapid, causing the temperature inside the compost pile to rise quickly. The microbes involved at this stage tend to be those that predominate in the soil. One example is **Actinomyces**, which resemble fungi but which are actually bacteria composed of filaments. They are what give the soil its earthy smell. **Enzymes** in Actinomyces are capable of degrading grass, bark and even newspaper. Species of fungi and **protozoa** can also be active at this stage.

As the internal temperature of the pile exceeds 40° C (104° F), the mesophiles die off and are replaced by the thermophilic microbes. A decomposition temperature around 55° C (131° F) is ideal, as microbial activity is pronounced and because that temperature is lethal to most human and animal microbial pathogens. Thus, the composting process is also a sterilizing process, from an infectious point of view. However, temperatures much above this point can kill off the microbes involved in the decomposition. For this reason,

compost piles are occasionally agitated or "turned over" to mix the contents, allow oxygen to diffuse throughout the material (efficient decomposition requires the presence of oxygen) and to disperse some of the heat. The ideal blend of microorganisms can be established and maintained by the addition of waste material to the compost pile so as to not let the pile become enriched in carbon or nitrogen. A proper ratio is about 30 parts carbon to one part nitrogen by weight.

Thermophilic bacteria present at this stage of decomposition include *Bacillus stearothermophilus* and bacteria of the genus Thermus. A variety of thermophilic fungi are present as well. These include *Rhizomucor pusills*, *Chaetomium thermophile*, *Humicola insolens*, *Humicola lanuginosus*, *Thermoascus aurabtiacus*, and *Aspergillus fumigatus*.

Thermophilic activity decomposes protein, fat, and carbohydrates such as the cellulose that makes up plants and grass. As this phase of decomposition ends, the temperature drops and once again the lower-temperature microbes become dominant. The decomposition of the complex materials by the thermophilic organisms provides additional nutrients for the continued decomposition by the mesophilic populations.

Microbiological composting is becoming increasingly important as space for waste disposal becomes limited. Some 30% of yard and household waste in the United States is compostable. An average household can decompose about 700 pounds of material per year. If such waste is added to landfills intact, the subsequent decomposition produces methane gas and acidic run-off, both of which are environmentally undesirable.

See also Chemoautotrophic and chemolithotrophic bacteria; Economic uses and benefits of microorganisms; Soil formation, involvement of microorganisms

COMPOUND MICROSCOPE • *see* MICROSCOPE AND MICROSCOPY

CONDITIONAL LETHAL MUTANT • *see* MICROBIAL GENETICS

CONFIRMED TESTS • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

CONFOCAL MICROSCOPY • *see* MICROSCOPE AND MICROSCOPY

CONJUGATION

Conjugation is a mechanism whereby a bacterium can transfer genetic material to an adjacent bacterium. The genetic transfer requires contact between the two **bacteria**. This contact is mediated by the bacterial appendage called a pilus.

Conjugation allows bacteria to increase their genetic diversity. Thus, an advantageous genetic trait present in a bacterium is capable of transfer to other bacteria. Without conjugation, the normal bacterial division process does not allow for the sharing of genetic information and, except for **mutations** that occur, does not allow for the development of genetic diversity.

A pilus is a hollow tube constructed of a particular protein. One end is anchored to the surface of a bacterium. The other end is capable of binding to specific proteins on the surface of another bacterium. A pilus can then act as a portal from the **cytoplasm** of one bacterium to the cytoplasm of the other bacterium. How the underlying membrane layers form channels to the bacterial cytoplasm is still unclear, although channel formation may involve what is termed a mating pair formation (mpf) apparatus on the bacterial surface.

Nonetheless, once a channel has been formed, transfer of **deoxyribonucleic acid** (**DNA**) from one bacterium (the donor) to the other bacterium (the recipient) can occur.

Conjugation requires a set of F (fertility) genes. Transfer of DNA from the genome of a bacterium can occur if the F set of genes is integrated in the bacterial chromosome. These F genes enter the pilus and literally drag the trailing genome along behind. Often the pilus will break before the transfer of the complete genome can occur. Thus, genes that are located in the vicinity of the F genes will tend to be successfully transferred in conjugation more often than genes located far away from the F genes. This process was originally discovered in ***Escherichia coli***. Strains that exhibit a higher than usual tendency to transfer genomic DNA are known as High Frequency of **Recombination** (Hfr) strains.

Conjugation also involves transfer of DNA that is located on a plasmid. A plasmid that contains the F genes is called the F episome or F plasmid. Other genes on the episome will be transferred very efficiently, since the entire episome can typically be transferred before conjugation is terminated by pilus breakage. If one of the genes codes for a disease causing factor or **antibiotic resistance** determinant, then episomal conjugation can be a powerful means of spreading the genetic trait through a bacterial population. Indeed, conjugation is the principle means by which bacterial antibiotic resistance is spread.

Finally, conjugation can involve the transfer of only a plasmid containing the F genes. This type of conjugation is also an efficient means of spreading genetic information to other bacteria. In this case, as more bacteria acquire the F genes, the proportion of the population that is capable of genetic transfer via conjugation increases.

Joshua Lederberg discovered the process of conjugation in 1945. He experimented with so-called nutritional **mutants** (bacteria that required the addition of a specific nutrient to the growth medium). By incubating the nutritional mutants in the presence of bacteria that did not require the nutrient to be added, Lederberg demonstrated that the mutation could be eliminated. Subsequently, another bacteriologist, William Hayes, demonstrated that the acquisition of genetic information occurred in a one-way manner (e.g., information was passing from one bacterium into another), and that the basis for the information transfer was genetic (i.e., mutants were isolated in which the transfer did not occur).

Another landmark experiment in microbiology also centered on conjugation. This experiment is known as the interrupted mating experiment (or blender experiment, since a common kitchen blender was used). Donor and recipient bacteria were mixed together and left to allow conjugation to begin. Then, at various times, the population was vigorously blended. This sheared off the pili that were connected the conjugating bacteria, interrupting the mating process. By analyzing the recipient bacteria for the presence of known genes that has been transferred, the speed of conjugation could be measured.

Conjugation has been exploited in the **biotechnology** era to permit the transfer of desired genetic information. A target **gene** can be inserted into the donor bacterial DNA near the F genes. Or, an F plasmid can be constructed in the laboratory and then inserted into a bacterial strain that will function as the donor. When conjugation occurs, bacteria in the recipient population will acquire the target gene.

See also Evolution and evolutionary mechanisms; Laboratory techniques in microbiology

CONTAMINATION AND RELEASE PREVENTION PROTOCOL

Contamination is the unwanted presence of a microorganism in a particular environment. That environment can be in the laboratory setting, for example, in a medium being used for the growth of a species of **bacteria** during an experiment. Another environment can be the human body, where contamination of various niches can produce an infection. Still another environment can be the solid and liquid nutrients that sustain life. A final example, which is becoming more relevant since the burgeoning use of **biotechnology**, is the natural environment. The consequences of the release of bioengineered **microorganisms** into the natural environment to the natural microflora and to other species that depend on the environment for their welfare, are often unclear.

The recognition of the adverse effects of contamination have been recognized for a long time, and steps that are now a vital part of microbiological practice were developed to curb contamination. The prevention of microbial contamination goes hand in hand with the use of microorganisms.

Ever since the development of techniques to obtain microorganisms in pure **culture**, the susceptibility of such cultures to the unwanted growth of other microbes has been recognized. This contamination extends far beyond being merely a nuisance. Differing behaviors of different microorganisms, in terms of how nutrients are processed and the by-products of this **metabolism**, can compromise the results of an experiment, leading to erroneous conclusions.

In the medical setting, microbial contamination can be life threatening. As recognized by **Joseph Lister** in the mid-nineteenth century, such contamination can be lessened, if not prevented completely, by the observance of various hygienic practices in the hospital setting. In modern medicine and science, the importance of hand washing and the maintenance of a sterile operating theatre is taken for granted.

Prevention of microbiological contamination begins in the laboratory. A variety of prevention procedures are a common part of an efficient microbiology laboratory. The use of sterile equipment and receptacles for liquid and solid growth media is a must. The prevention of contamination during the manipulations of microorganisms in the laboratory falls under the term asceptic technique. Examples of asceptic technique include the **disinfection** of work surfaces and the hands of the relevant lab personnel before and after contact with the microorganisms and the flaming of the metal loops or rods used to transfer bacter from one location to another.

In other areas of a laboratory, microorganisms that are known to be of particular concern, because they can easily contaminate or be contaminated, or because they represent a health threat, can be quarantined in special work areas. Examples of such areas include fume hoods and the so-called glove box. The latter is an enclosed space where the lab worker is kept physically separate from the microorganisms, but can manipulate the organisms by virtue of rubber gloves that are part of the wall of the enclosure.

In both the laboratory and other settings, such as processing areas for foods, various monitoring steps are instituted as part of a proper quality control regimen to ensure that contamination does not occur, or can be swiftly detected and dealt with. A well-established technique of contamination monitoring is the air plate technique, where a non-specific growth medium is exposed to the circulating air in the work area for a pre-determined period of time. Air-borne microorganisms can be detected in this manner. More recently, as the importance of the adherent (biofilm) mode of growth of, in particular, bacteria became recognized, contamination monitoring can also include the installation of a device that allows the fluid circulating through pipelines to be monitored. Thus, for example, water used in processing operations can be sampled to determine if **bacterial growth** on the pipeline is occurring and also whether remediation is necessary.

A necessary part of the prevention of microbiological contamination is the establishment of various quality control measures. For example, the swiping of a lab bench with a sterile cotton swab and the incubation of the swab in a nonspecific growth medium is a regular part of many microbiology laboratories quality control regimen. The performance of all equipment that is used for **sterilization** and microorganism confinement is also regularly checked.

With the advent of biotechnology and in particular the use of genetically modified microorganisms in the agricultural sector, the prevention of the unwanted release of the bioengineered microbes into the natural environment has become an important issue to address.

The experimentation with genetically engineered microorganisms in the natural environment is subject to a series of rigid controls in many countries around the world. A series of benchmarks must be met to ensure that an organism is either incapable of being spread or, if so, is incapable of prolonged survival.

Firefighters remove barrel conaining suspected infectious agent.

Prevention of genetic contamination, via the exchange of genetic material between the bioengineered microbe and the natural microbial population, is difficult to prevent. However, available evidence supports the view that the genetic traits bred into the bioengineered organism to permit its detection, such as **antibiotic resistance**, are not traits that will be maintained in the natural population. This is because of the energy cost to the microorganism to express the trait and because of the mathematical dynamics of population genetics (i.e., the altered genes are not present in numbers to become established within the greater population) and the absence of the need for the trait (the antibiotic of interest is not present in the natural environment). Hence, contamination prevention procedures have tended to focus on those aspects of contamination that are both relevant and likely to occur.

As an example of the measures currently in place, the United States has three agencies that are concerned with the regulation of biotechnology. These are the Department of Agriculture, Environmental Protection Agency, and the Food and Drug Administration. Each of these agencies oversee regulatory legislation that addresses the contamination of various natural and commercially relevant environments.

See also Asilomar conferences; Biotechnology; Hazard Analysis and Critical Point Program (HACPP); Laboratory techniques in microbiology

CONTAMINATION, BACTERIAL AND VIRAL

Contamination by **bacteria** and **viruses** can occur on several levels. In the setting of the laboratory, the growth media, tissues and other preparations used for experimentation can support the growth of unintended and unwanted **microorganisms**. Their presence can adversely influence the results of the experiments. Outside the laboratory, bacteria and viruses can contaminate drinking water supplies, foodstuffs, and products, causing illness. Infection is another form of contamination.

Equipment and growth media used in the laboratory must often be treated to render them free of microorganisms. Bacteria and viruses can be present in the air, as aerosolized droplets, and can be present on animate surfaces, such as the skin and the mucous membranes of the nasal passage, and on inanimate surfaces, such as the workbenches in the laboratory. Without precautions and the observance of what is known as sterile technique, these microbes can contaminate laboratory growth media, solutions and equipment. This contamination can be inconvenient, necessitating the termination of an experiment. However, if the contamination escapes the notice of the researcher, then the results obtained will be unknowingly marred. Whole avenues of research could be compromised.

Contamination of drinking water by bacteria and viruses has been a concern since antiquity. Inadequate sanitation practices can introduce fecal material into the water. Enteroviruses and fecal bacteria such as *Shigella* and ***Escherichia coli*** O157:H7 are capable of causing debilitating, even life-threatening, diseases. Even in developed countries, contamination of drinking water remains a problem. If a treatment system is not functioning properly, water sources, especially surface sources, are vulnerable to contamination. An example occurred in the summer of 2000 in Walkerton, Ontario, Canada. Contamination of one of the town's wells by *Escherichia coli* O157:H7 run-off from a cattle operation killed seven people, and sickened over two thousand.

Other products can be contaminated as well. An example is blood and blood products. Those who donate blood might be infected, and the infectious agents can be transmitted to the recipient of the blood or blood product. In the 1970s and 1980s, the Canadian blood supply was contaminated with the viral agents of **hepatitis** and acquired **immunodeficiency** syndrome. At that time, tests for these agents were not as sophisticated and as definitive as they are now. The viruses that escaped detection sickened thousands of people. Blood supplies in Canada and elsewhere are now safeguarded from contamination by stringent monitoring programs.

Food products are also prone to contamination. The contamination can originate in the breeding environment. For example, poultry that are grown in crowded conditions are reservoirs of bacterial contamination, particularly with *Campylobacter jejuni*. Over half of all poultry entering processing plants are contaminated with this bacterium. Other food products can become contaminated during processing, via bacteria that are growing on machinery or in processing solutions. Quality control measures, which monitor critical phases of the process from raw material to finished product, are helpful in pinpointing and eliminating sources of contamination.

Typical anti-contamination garb worn during a surgical procedure.

With respect to contamination of food, the **hygiene** of food handlers is a key factor. In the United States, estimates are the one in five food-borne disease outbreaks is caused by the handling of foods by personnel whose hands are contaminated with bacteria or viruses. Poor hand washing following use of the bathroom is the main problem.

In the nineteenth century, similar hygiene problems created a death rate in most surgical procedures. The contamination of open wounds, incisions and entry routes of catheters killed the majority of surgical patients. With the adoption of sterile operating room technique and scrupulous personnel hygiene, the death rate from surgical procedures is now very low.

See also Blood borne infections; Hazard Analysis and Critical Point Program (HAACP); History of public health; Transmission of pathogens

Coryneform bacteria

Coryneform **bacteria** are normal residents of the skin. They can also cause opportunistic infections. These are infections that occur as a secondary infection, when the immune response of a host has been weakened by another infection or by another insult to the **immune system**, such as **chemotherapy**. An example is *Corynebacterium jeikeium*, whose infection can be taxing to treat as the organism is resistant to numerous **antibiotics**. Over the past two decades, the numbers of such infections have been rising. This may be an indication of an immune stress on the body.

Coryneform bacteria can stain positive in the Gram stain protocol. However, this reaction is not consistent. A characteristic feature is their tendency to arrange themselves in a V-like pattern or lined up, much like logs stacked one against the other.

While there are some consistencies among the members of the Coryneform bacteria, a hallmark of these bacteria is their diversity of habitats. This, and their inconsistent Gram stain reaction, can make identification of the microorganism tedious. More rigorous biochemical and molecular biological tools of identification are being used by organizations such as the **Centers for Disease Control** (CDC) to establish a definitive classification scheme for Coryneform bacteria. For example, CDC groups JK and D-2 in the genus *Corynebacterium* are now recognized as important human disease-causing **microorganisms**. Conversely, these rigorous techniques have resulted in the removal of some species of bacteria from the genus.

Coryneform bacteria are important medically. *Corynebacterium diphtheriae* is the organism that causes **diphtheria**. In fact, before Coryneform bacteria were known to

be comprised of several species, the bacteria were referred to as diphtheroids. Diphtheria is apparent as an **inflammation** and bleeding of the throat and as a generalized toxic poisoning of the body, due to the release of a powerful toxin by the bacteria. The toxin spreads throughout the body via the bloodstream and has a particular affinity for tissues such as the heart, nerve endings and the adrenal glands. Diphtheria is treatable with antibiotics.

Other species of the genus *Corynebacterium* cause mastitis in cows (an infection and inflammation of the udder), infection of the lymph nodes of sheep, and skin rashes and ulcerations in humans. *Rhodococcus equi*, which inhabits soil, is an important pathogen of young horses. Another human pathogen is It is also a normal resident on skin surfaces, and can cause an infection in those receiving chemotherapy.

See also Gram staining

COSTERTON, JOHN WILLIAM (1934-)

Canadian microbiologist

J. William (Bill) Costerton is a Canadian microbiologist who has pioneered the recognition of bacterial **biofilms** as the dominant mode of growth of **bacteria**, and who first demonstrated their importance in the resistance of bacteria to antibacterial agents and the persistence of some chronic bacterial infections.

Costerton was born in Vernon, British Columbia. His early education was in that province. In 1955, he received a B.S. in bacteriology and **immunology** from the University of British Columbia, followed by a M.S. in the same discipline from UBC in 1956. He then studied in the laboratory of Dr. **Robert Murray** at the University of Western Ontario in London, Ontario, where he received a Ph.D. in 1960. Following postdoctoral training at Cambridge University, Costerton moved to MacDonald College of McGill University, in the Canadian province of Quebec, where he became first a Professional Associate in 1966 then an Assistant Professor in 1968. In 1970 he moved to the University of Calgary as an Associate Professor. He became a tenured Professor at Calgary in 1975. From 1985 to 1992, he held positions at Calgary as the AOSTRA Research Professor followed by the National Sciences and Engineering Research Council Industrial Research Chair. These two appointments freed him from teaching to concentrate on his burgeoning research into bacterial biofilms.

Research on biofilms has occupied Costerton since his move to Calgary. Costerton and his colleagues demonstrated the existence of biofilms and when on to show that biofilms are the dominant mode of growth for bacteria. The elaboration of an extensive sugar network that adheres bacteria to surfaces and subsequently buries them was revealed. Research over a decade demonstrated the importance of this exopolysaccharide in enabling the bacteria to survive doses of antibacterial agents, including **antibiotics** that readily killed bacteria grown in conventional lab cultures. This research was so convincing that an initially skeptical scientific community became convinced of the importance and widespread nature of biofilms.

In 1993, Costerton left Calgary to take up the post of Director of the Center for Biofilm Engineering at Montana State University, Bozeman. Since then, he and his colleagues have used techniques such as confocal microscopy to probe intact biofilms without disrupting them. These studies have revealed the complex nature of biofilm structure and the coordinated nature of the interaction between the bacterial populations in the biofilms. As well, Costerton discovered the so-called bioelectric effect, in which an application of current makes a biofilm much more susceptible to antibiotic killing. These discoveries are having profound influence on the design of strategies to combat chronic infections, such as the *Pseudomonas aeruginosa* lung infections that occur, and can ultimately kill those afflicted with cystic fibrosis.

For these and other pioneering contributions to biofilm research, Costerton has received many awards. These include the Sir Frederick Haultain Prize for outstanding achievement in the physical sciences (1986), the Isaak Walton Killam Memorial Prize for Scientific Achievement in Canada (1990), and a Fellowship in the American Association for the Advancement of Science (1997).

Costerton continues his research at Montana State and is actively involved internationally in promoting the multidisciplinary structure of the Center's research and education curriculum.

See also Antibiotic resistance, tests for; Bacterial adaptation; Glycocalyx

COULTER COUNTER

A Coulter counter is a device that is used to measure the number of cells in a certain volume of a sample suspension. The counter achieves this enumeration by monitoring the decrease in electrical conductivity that occurs when the cells pass through a small opening in the device. While originally developed for use with blood cells, the Coulter counter has found great use in a diverse number of disciplines, including microbiology, where it is used to determine the total number of **bacteria** in samples.

Because the device operates on the physical blockage of electrical conductivity by particles in a sample, the Coulter counter cannot distinguish between living and dead bacteria. An indication of the total number of bacteria (alive, dormant, and dead) is provided. The number of living bacteria can, however, usually be easily determined using another volume from the same sample (e.g., the heterotrophic plate count).

The Coulter counter is named after its inventor. Wallace H. Coulter conceived and constructed the first counter in the basement of his home in Chicago in the early 1950s. Then as now, the device relies on a vacuum pump that draws a solution or suspension through an electrically charged tube that has a tiny hole at the other end. As particles pass through the hole the electrical field is interrupted. The pattern of the interruption can be related to the number of particles and even to particle type (e.g., red blood cell versus bacteria).

A bacterial suspension is best analyzed in the Coulter counter when the suspension has been thoroughly shaken beforehand. This step disperses the bacteria. Most bacteria tend to aggregate together in a suspension. If not dispersed, a clump of bacteria passing through the orifice of the counter could be counted as a single bacterium. This would produce an underestimate of the number of bacteria in the suspension.

The Coulter counter has been used for many applications, both biological and nonbiological. In the 1970s, the device was reconfigured to incorporate a laser beam. This allowed the use of fluorescent labeled monoclonal antibodies to detect specific types of cells (e.g., cancer cells) or to detect a specific species of bacteria. This refinement of the Coulter counter is now known as flow cytometry.

See also Bacterial growth and division; Laboratory techniques in microbiology

COWPOX

Cowpox refers to a disease that is caused by the cowpox or catpox virus. The virus is a member of the orthopoxvirus family. Other **viruses** in this family include the **smallpox** and vaccinia viruses. Cowpox is a rare disease, and is mostly noteworthy as the basis of the formulation, over 200 years ago, of an injection by **Edward Jenner** that proved successful in curing smallpox.

The use of cowpox virus as a means of combating smallpox, which is a much more threatening disease to humans, has remained popular since the time of Jenner.

Once a relatively common malady in humans, cowpox is now confined mostly to small mammals in Europe and the United Kingdom. The last recorded case of a cow with cowpox was in the United Kingdom in 1978. Occasionally the disease is transmitted from these sources to human. But this is very rare. Indeed, only some 60 cases of human cowpox have been reported in the medical literature.

The natural reservoir for the cowpox virus is believed to be small woodland animals, such as voles and wood mice. Cats and cows, which can harbor the virus, are thought to be an accidental host, perhaps because of their contact with the voles or mice.

The cowpox virus, similar to the other orthopoxvirus, is best seen using the **electron microscopic** technique of negative staining. This technique reveals surface details. The cowpox virus is slightly oval in shape and has a very ridged-appearing surface.

Human infection with the cowpox virus is thought to require direct contact with an infected animal. The virus gains entry to the bloodstream through an open cut. In centuries past, farmers regularly exposed to dairy cattle could acquire the disease from hand milking the cows, for example. Cowpox is typically evident as pus-filled sores on the hands and face that subsequently turn black before fading away. While present, the lesions are extremely painful. There can be scars left at the site of the infection. In rare instances, the virus can become more widely disseminated through the body, resulting in death.

Both males and females are equally as likely to acquire cowpox. Similarly, there no racial group is any more susceptible to infection. There is a predilection towards acquiring the infection in youth less than 18 years of age. This may be because of a closer contact with animals such as cats by this age group, or because of lack of administration of smallpox **vaccine**.

Treatment for cowpox tends to be ensuring that the patient is as comfortable as possible while waiting for the infection to run its course. Sometimes, a physician may wish to drain the pus from the skin sores to prevent the spread of the infection further over the surface of the skin. In cases where symptoms are more severe, an immune globulin known as antivaccinia gamaglobulin may be used. This immunoglobulin is reactive against all viruses of the orthopoxvirus family. The use of this treatment needs to be evaluated carefully, as there can be side effects such as kidney damage. Antibodies to the vaccinia virus may also be injected into a patient, as these antibodies also confer protection against cowpox.

See also Vaccination; Virology; Zoonoses

COXIELLA BURNETII • *see* Q FEVER

CRANBERRY JUICE AS AN ANTI-ADHESION METHOD • *see* ANTI-ADHESION METHODS

CREUTZFELDT-JAKOB DISEASE (CJD) • *see* BSE AND CJD DISEASE

CRICK, FRANCIS (1916-)

English molecular biologist

Francis Crick is one half of the famous pair of molecular biologists who unraveled the mystery of the structure of **DNA** (**deoxyribonucleic acid**), the carrier of genetic information, thus ushering in the modern era of **molecular biology**. Since this fundamental discovery, Crick has made significant contributions to the understanding of the **genetic code** and **gene** action, as well as the understanding of molecular neurobiology. In Horace Judson's book *The Eighth Day of Creation,* Nobel laureate **Jacques Lucien Monod** is quoted as saying, "No one man created molecular biology. But Francis Crick dominates intellectually the whole field. He knows the most and understands the most." Crick shared the Nobel Prize in medicine in 1962 with **James Watson** and **Maurice Wilkins** for the elucidation of the structure of DNA.

The eldest of two sons, Francis Harry Compton Crick was born to Harry Crick and Anne Elizabeth Wilkins in Northampton, England. His father and uncle ran a shoe and boot factory. Crick attended grammar school in Northampton, and was an enthusiastic experimental scientist at an early age, producing the customary number of youthful chemical explo-

Francis Crick (right) and James Watson (left), who deduced the structure of the DNA double helix (shown between them).

sions. As a schoolboy, he won a prize for collecting wildflowers. In his autobiography, *What Mad Pursuit,* Crick describes how, along with his brother, he "was mad about tennis," but not much interested in other sports and games. At the age of fourteen, he obtained a scholarship to Mill Hill School in North London. Four years later, at eighteen, he entered University College, London. At the time of his matriculation, his parents had moved from Northampton to Mill Hill, and this allowed Crick to live at home while attending university. Crick obtained a second-class honors degree in physics, with additional work in mathematics, in three years. In his autobiography, Crick writes of his education in a rather light-hearted way. Crick states that his background in physics and mathematics was sound, but quite classical, while he says that he learned and understood very little in the field of chemistry. Like many of the physicists who became the first molecular

biologists and who began their careers around the end of World War II, Crick read and was impressed by Erwin Schrödinger's book *What Is Life?,* but later recognized its limitations in its neglect of chemistry.

Following his undergraduate studies, Crick conducted research on the viscosity of water under pressure at high temperatures, under the direction of Edward Neville da Costa Andrade, at University College. It was during this period that he was helped financially by his uncle, Arthur Crick. In 1940, Crick was given a civilian job at the Admiralty, eventually working on the design of mines used to destroy shipping. Early in the year, Crick married Ruth Doreen Dodd. Their son Michael was born during an air raid on London on November 25, 1940. By the end of the war, Crick was assigned to scientific intelligence at the British Admiralty Headquarters in Whitehall to design weapons.

Realizing that he would need additional education to satisfy his desire to do fundamental research, Crick decided to work toward an advanced degree. Crick became fascinated with two areas of biology, particularly, as he describes it in his autobiography, "the borderline between the living and the nonliving, and the workings of the brain." He chose the former area as his field of study, despite the fact that he knew little about either subject. After preliminary inquiries at University College, Crick settled on a program at the Strangeways Laboratory in Cambridge under the direction of Arthur Hughes in 1947, to work on the physical properties of **cytoplasm** in cultured chick fibroblast cells. Two years later, he joined the Medical Research Council Unit at the Cavendish Laboratory, ostensibly to work on protein structure with British chemists Max Perutz and John Kendrew (both future Nobel Prize laureates), but eventually to work on the structure of DNA with Watson.

In 1947, Crick was divorced, and in 1949, married Odile Speed, an art student whom he had met during the war. Their marriage coincided with the start of Crick's Ph.D. thesis work on the x-ray diffraction of proteins. X-ray diffraction is a technique for studying the crystalline structure of molecules, permitting investigators to determine elements of three-dimensional structure. In this technique, x rays are directed at a compound, and the subsequent scattering of the x-ray beam reflects the molecule's configuration on a photographic plate.

In 1941 the Cavendish Laboratory where Crick worked was under the direction of physicist Sir William Lawrence Bragg, who had originated the x-ray diffraction technique forty years before. Perutz had come to the Cavendish to apply Bragg's methods to large molecules, particularly proteins. In 1951, Crick was joined at the Cavendish by James Watson, a visiting American who had been trained by Italian physician Salvador Edward Luria and was a member of the Phage Group, a group of physicists who studied bacterial **viruses** (known as bacteriophages, or simply phages). Like his phage colleagues, Watson was interested in discovering the fundamental substance of genes and thought that unraveling the structure of DNA was the most promising solution. The informal partnership between Crick and Watson developed, according to Crick, because of their similar "youthful arrogance" and similar thought processes. It was also clear that their experiences complemented one another. By the time of their first meeting, Crick had taught himself a great deal about x-ray diffraction and protein structure, while Watson had become well informed about phage and bacterial genetics.

Both Crick and Watson were aware of the work of biochemists Maurice Wilkins and Rosalind Franklin at King's College, London, who were using x-ray diffraction to study the structure of DNA. Crick, in particular, urged the London group to build models, much as American chemist Linus Pauling had done to solve the problem of the alpha helix of proteins. Pauling, the father of the concept of the chemical bond, had demonstrated that proteins had a three-dimensional structure and were not simply linear strings of amino acids. Wilkins and Franklin, working independently, preferred a more deliberate experimental approach over the theoretical, model-building scheme used by Pauling and advocated by Crick. Thus, finding the King's College group unresponsive to their suggestions, Crick and Watson devoted portions of a two-year period discussing and arguing about the problem. In early 1953, they began to build models of DNA.

Using Franklin's x-ray diffraction data and a great deal of trial and error, they produced a model of the DNA molecule that conformed both to the London group's findings and to the data of Austrian-born American biochemist Erwin Chargaff. In 1950, Chargaff had demonstrated that the relative amounts of the four nucleotides, or bases, that make up DNA conformed to certain rules, one of which was that the amount of adenine (A) was always equal to the amount of thymine (T), and the amount of guanine (G) was always equal to the amount of cytosine (C). Such a relationship suggests pairings of A and T, and G and C, and refutes the idea that DNA is nothing more than a tetranucleotide, that is, a simple molecule consisting of all four bases.

During the spring and summer of 1953, Crick and Watson wrote four papers about the structure and the supposed function of DNA, the first of which appeared in the journal *Nature* on April 25. This paper was accompanied by papers by Wilkins, Franklin, and their colleagues, presenting experimental evidence that supported the Watson-Crick model. Watson won the coin toss that placed his name first in the authorship, thus forever institutionalizing this fundamental scientific accomplishment as "Watson-Crick."

The first paper contains one of the most remarkable sentences in scientific writing: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." This conservative statement (it has been described as "coy" by some observers) was followed by a more speculative paper in *Nature* about a month later that more clearly argued for the fundamental biological importance of DNA. Both papers were discussed at the 1953 Cold Spring Harbor Symposium, and the reaction of the developing community of molecular biologists was enthusiastic. Within a year, the Watson-Crick model began to generate a broad spectrum of important research in genetics.

Over the next several years, Crick began to examine the relationship between DNA and the genetic code. One of his first efforts was a collaboration with Vernon Ingram,

which led to Ingram's 1956 demonstration that sickle cell hemoglobin differed from normal hemoglobin by a single amino acid. Ingram's research presented evidence that a molecular genetic disease, caused by a Mendelian mutation, could be connected to a DNA-protein relationship. The importance of this work to Crick's thinking about the function of DNA cannot be underestimated. It established the first function of "the genetic substance" in determining the specificity of proteins.

About this time, South African-born English geneticist and molecular biologist **Sydney Brenner** joined Crick at the Cavendish Laboratory. They began to work on the coding problem, that is, how the sequence of DNA bases would specify the amino acid sequence in a protein. This work was first presented in 1957, in a paper given by Crick to the Symposium of the Society for Experimental Biology and entitled "On Protein Synthesis." Judson states in *The Eighth Day of Creation* that "the paper permanently altered the logic of biology." While the events of the **transcription** of DNA and the synthesis of protein were not clearly understood, this paper succinctly states "The Sequence Hypothesis... assumes that the specificity of a piece of nucleic acid is expressed solely by the sequence of its bases, and that this sequence is a (simple) code for the amino acid sequence of a particular protein." Further, Crick articulated what he termed "The Central Dogma" of molecular biology, "that once 'information' has passed into protein, it cannot get out again. In more detail, the transfer of information from nucleic acid to nucleic acid, or from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible." In this important theoretical paper, Crick establishes not only the basis of the genetic code but predicts the mechanism for **protein synthesis**. The first step, transcription, would be the transfer of information in DNA to **ribonucleic acid** (**RNA**), and the second step, **translation**, would be the transfer of information from RNA to protein. Hence, the genetic message is transcribed to a messenger, and that message is eventually translated into action in the synthesis of a protein. Crick is credited with developing the term "codon" as it applies to the set of three bases that code for one specific amino acid. These codons are used as "signs" to guide protein synthesis within the cell.

A few years later, American geneticist Marshall Warren Nirenberg and others discovered that the nucleic acid sequence U-U-U (polyuracil) encodes for the amino acid phenylalanine, and thus began the construction of the DNA/RNA dictionary. By 1966, the DNA triplet code for twenty amino acids had been worked out by Nirenberg and others, along with details of protein synthesis and an elegant example of the control of protein synthesis by French geneticist **François Jacob**, Arthur Pardée, and French biochemist Jacques Lucien Monod. Brenner and Crick themselves turned to problems in developmental biology in the 1960s, eventually studying the structure and possible function of histones, the class of proteins associated with **chromosomes**.

In 1976, while on sabbatical from the Cavendish, Crick was offered a permanent position at the Salk Institute for Biological Studies in La Jolla, California. He accepted an endowed chair as Kieckhefer Professor and has been at the Salk Institute ever since. At the Salk Institute, Crick began to study the workings of the brain, a subject that he had been interested in from the beginning of his scientific career. While his primary interest was consciousness, he attempted to approach this subject through the study of vision. He published several speculative papers on the mechanisms of dreams and of attention, but, as he stated in his autobiography, "I have yet to produce any theory that is both novel and also explains many disconnected experimental facts in a convincing way."

During his career as an energetic theorist of modern biology, Francis Crick has accumulated, refined, and synthesized the experimental work of others, and has brought his unusual insights to fundamental problems in science.

See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Genetic identification of microorganisms; Genetic mapping; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Genotype and phenotype; Immunogenetics

CRYOPROTECTION

Cryopreservation refers to the use of a very low temperature (below approximately –130° C [–202° F]) to store a living organism. Organisms (including many types of **bacteria**, **yeast**, **fungi**, and algae) can be frozen for long periods of time and then recovered for subsequent use.

This form of long-term storage minimizes the chances of change to the microorganism during storage. Even at refrigeration temperature, many **microorganisms** can grow slowly and so might become altered during storage. This behavior has been described for strains of *Pseudomonas aeruginosa* that produce an external slime layer. When grown on a solid **agar** surface, the colonies of such strains appear like mucous drops. However, when recovered from refrigeration storage, the mucoid appearance can be lost. Cryopreservation of mucoid strains maintains the mucoid characteristic.

Cryostorage of bacteria must be done at or below the temperature of –130° C [–202° F], as it is at this temperature that frozen water can form crystals. Because much of the interior of a bacterium and much of the surrounding membrane(s) are made of water, crystal formation would be disastrous to the cell. The formation of crystals would destroy structure, which would in turn destroy function.

Ultralow temperature freezers have been developed that achieve a temperature of –130° C . Another popular option for cryopreservation is to immerse the sample in a compound called liquid nitrogen. Using liquid nitrogen, a temperature of –196° C [–320.8° F] can be achieved.

Another feature of bacteria that must be taken into account during cryopreservation is called osmotic pressure. This refers to the balance of ions on the outside versus the inside of the cell. An imbalance in osmotic pressure can cause water to flow out of or into a bacterium. The resulting shrinkage or ballooning of the bacterium can be lethal.

To protect against crystal formation and osmotic pressure shock to the bacteria, bacterial suspensions are typically prepared in a so-called cryoprotectant solution. Glycerol is an effective cryoprotective agent for many bacteria. For other bacteria, such as cyanobacteria, methanol and dimethyl sulfoxide are more suitable.

The microorganisms used in the cryoprotection process should be in robust health. Bacteria, for example, should be obtained from the point in their growth cycle where they are actively growing and divided. In conventional liquid growth media, this is described as the mid-logarithmic phase of growth. In older cultures, where nutrients are becoming depleted and waste products are accumulating, the cells can deteriorate and change their characteristics.

For bacteria, the cryoprotectant solution is added directly to an agar **culture** of the bacteria of interest and bacteria are gently dislodged into the solution. Alternately, bacteria in a liquid culture can be centrifuged and the "pellet" of bacteria resuspended in the cryoprotectant solution. The resulting bacterial suspension is then added to several specially designed cryovials. These are made of plastic that can withstand the ultralow temperature.

The freezing process is done as quickly as possible to minimize crystal formation. This is also referred to as "snap freezing." Bacterial suspensions in t cryoprotectant are initially at room temperature. Each suspension is deep-frozen in a step-wise manner. First, the suspensions are chilled to refrigerator temperature. Next, they are stored for a few hours at –70° C [–94° F]. Finally, racks of cryovials are either put into the ultralow temperature freezer or plunged into liquid nitrogen. The liquid nitrogen almost instantaneously brings the samples to –196° C [–320.8° F]. Once at this point, the samples can be stored indefinitely.

Recovery from cryostorage must also be rapid to avoid crystal formation. Each suspension is warmed rapidly to room temperature. The bacteria are immediately recovered by centrifugation and the pellet of bacteria is resuspended in fresh growth medium. The suspension is allowed to adapt to the new temperature for a few days before being used.

Cryoprotection can be used for other purposes than the long-term storage of samples. For example, cryoelectron microscopy involves the rapid freezing of a sample and examination of portions of the sample in an electro **microscope** under conditions where the ultralow temperature is maintained. If done correctly, cryoelectron microscopy will revel features of microorganisms that are not otherwise evident in conventional electron microscopy. For example, the watery **glycocalyx**, which is made of chains of sugar, collapses onto the surface of a bacterium as the sample is dried out during preparation for conventional electron microscopy. But glycocalyx structure can be cryopreserved. In another example, cryoelectron microscopy has also maintained external structural order on virus particles, allowing researchers to deduce how these structures function in the viral infection of tissue.

See also Bacterial ultrastructure; Donnan equilibrium; Quality control in microbiology

CRYPTOCOCCI AND CRYPTOCOCCOSIS

Cryptococcus is a **yeast** that has a capsule surrounding the cell. In the yeast classification system, *Cryptococcus* is a member of the Phylum Basidimycota, Subphylum Basidimycotina, Order Sporidiales, and Family Sporidiobolaceae.

There are 37 species in the genus *Cryptococcus*. One of these, only one species is disease-causing, *Cryptococcus neoformans*. There are three so-called varieties of this species, based on antigenic differences in the capsule, some differences in biochemical reactions such as the use of various sugars as nutrients, and in the shape of the spores produced by the yeast cells. The varieties are *Cryptococcus neoformans* var. *gatti*, *grubii*, and *neoformans*. The latter variety causes the most cryptococcal infections in humans.

Cryptococcus neoformans has a worldwide distribution. It is normally found on plants, fruits and in birds, such as pigeons and chicken. Transmission via bird waste is a typical route of human infection.

Cryptococcus neoformans causes an infection known as cryptococcosis. Inhalation of the microorganism leads to the persistent growth in the lungs. For those whose **immune system** is compromised, such as those having Acquired **Immunodeficiency** Syndrome (**AIDS**), the pulmonary infection can be life-threatening. In addition, yeast cells may become distributed elsewhere in the body, leading to **inflammation** of nerve lining in the brain (**meningitis**). A variety of other infections and symptoms can be present, including infections of the eye (conjunctivitis), ear (otitis), heart (myocarditis), liver (**hepatitis**), and bone (arthritis).

The most common illness caused by the cryptococcal fungus is cryptococcal meningitis. Those at most risk of developing cryptococcosis are AIDS patients. Those who have received an organ, are receiving **chemotherapy** for cancer or have Hodgkin's disease are also at risk, since frequently their immune systems are suppressed. As the incidence of AIDS and the use of immunosupressant drugs have grown over the past decade, the number of cases of cryptococcosis has risen. Until then, cases of cryptococcus occurred only rarely. Even today, those with a well-functioning immune system are seldom at risk for cryptococcosis. For these individuals a slight skin infection may be the only adverse effect of exposure to Cryptococcus.

Cryptococcus begins with the inhalation of *Cryptococcus neoformans*. Likely, the inhaled yeast is weakly encapsulated and is relatively small. This allows the cells to penetrate into the alveoli of the lungs. There the production of capsule occurs. The capsule surrounding each yeast cell aids the cell in avoiding the immune response of the host, particularly the engulfing of the yeast by macrophage cells (which is called **phagocytosis**). The capsule is comprised of chains of sugars, similar to the capsule around **bacteria**. The capsule of *Cryptococcus neoformans* is very negatively charged. Because cells such as macrophages are also negatively charged, repulsive forces will further discourage interaction of macrophages with the capsular material.

Another important virulence factor of the yeast is an enzyme called phenol oxidase. The enzyme operates in the

production of melanin. Current thought is that the phenol oxidase prevents the formation of charged hydroxy groups, which can be very damaging to the yeast cell. The yeast may actually recruit the body's melanin producing machinery to make the compound.

Cryptococcus neoformans also has other **enzymes** that act to degrade certain proteins and the **phospholipids** that make up cell membranes. These enzymes may help disrupt the host cell membrane, allowing the yeast cells penetrate into host tissue more easily.

Cryptococcus neoformans is able to grow at body temperature. The other Cryptococcus species cannot tolerate this elevated temperature.

Yet another virulence factor may operate. Evidence from laboratory studies has indicated that antigens from the yeast can induce a form of **T cells** that down regulates the immune response of the host. This is consistent with the knowledge that survivors of cryptococcal meningitis display a poorly operating immune system for a long time after the infection has ended. Thus, *Cryptococcus neoformans* may not only be capable of evading an immune response by the host, but may actually dampen down that response.

If the infection is treated while still confined to the lungs, especially in patients with a normally operative immune system, the prospects for full recovery are good. However, spread to the central nervous system is ominous, especially in immunocompromised patients.

The standard treatment for cryptococcal meningitis is the intravenous administration of a compound called amphotericin B. Unfortunately the compound has a raft of side effects, including fever, chills, headache, nausea with vomiting, diarrhea, kidney damage, and suppression of bone marrow. The latter can lead to a marked decrease in red blood cells. Studies are underway in which amphotericin B is enclosed in bags made of lipid material (called liposomes). The use of liposomes can allow the drug to be more specifically targeted to the site where treatment is most needed, rather than flooding the entire body with the drug. Hopefully, the use of liposome-delivered amphotericin B will lessen the side effects of therapy.

See also Fungi; Immunomodulation; Yeast, infectious

CRYPTOSPORIDIUM AND CRYPTOSPORIDIOSIS

Cryptosporidum is a protozoan, a single-celled parasite that lives in the intestines of humans and other animals. The organism causes an intestinal malady called cryptosporidiosis (which is commonly called "crypto").

The members of the genus *Cryptosporidium* infects epithelial cells, especially those that line the walls of the intestinal tract. One species, *Cryptosporidium muris*, infects laboratory tests species, such as rodents, but does not infect humans. Another species, *Cryptosporidium parvum*, infects a wide variety of mammals, including humans. Calculations have indicated that cattle alone release some five tons of the parasite each year in the United States alone.

Non-human mammals are the reservoir of the organism for humans. Typically, the organism is ingested when in water that has been contaminated with Cryptosporidium-containing feces. Often in an environment such as water, Cryptosporidium exists in a form that is analogous to a bacterial spore. In the case of Cryptosporidium, this dormant and environmentally resilient form is called an oocyst.

An oocyst is smaller than the growing form of Cryptosporidium. The small size can allow the oocyst to pass through some types of filters used to treat water. In addition, an oocyst is also resistant to the concentrations of chlorine that are widely used to disinfect drinking water. Thus, even drinking water from a properly operating municipal treatment plant has the potential to contain Cryptosporidium.

The organism can also be spread very easily by contact with feces, such as caring with someone with diarrhea or changing a diaper. Spread of cryptosporidiosis in nursing homes and day care facilities is not uncommon.

Only a few oocytes need to be ingested to cause cryptosporidiosis. Studies using volunteers indicate that an infectious dose is anywhere from nine to 30 oocysts. When an oocyte is ingested, it associates with intestinal epithelial cells. Then, four bodies called sporozoites, which are contained inside the oocyst, are released. These burrow inside the neighbouring epithelial cells and divide to form cells that are called merozoites. Eventually, the host cell bursts, releasing the merozoites. The freed cells go on to attack neighbouring epithelial cells and reproduce. The new progeny are released and the cycle continues over and over. The damage to the intestinal cells affects the functioning of the intestinal tract.

Cryptosporidium and its oocyte form have been known since about 1910. *Cryptosporidium parvum* was first described in 1911. Cryptosporidiosis has been a veterinary problem for a long time. The disease was recognized as a human disease in the 1970s. In the 1980s, the number of human cases rose sharply along with the cases of **AIDS**.

There have been many outbreaks of cryptosporidiosis since the 1980s. In 1987, 13,000 in Carrollton, Georgia contracted cryptosporidiosis via their municipal drinking water. This incident was the first case of the spread of the disease through water that had met all state and federal standards for microbiological quality. In 1993, an outbreak of cryptosporidiosis, again via contaminated municipal drinking water that met the current standards, sickened 400,000 people and resulted in several deaths. Outbreaks such as these prompted a change in **water quality** standards in the United States.

Symptoms of cryptosporidiosis are diarrhea, weight loss, and abdominal cramping. Oocysts are released in the feces all during the illness. Even when the symptoms are gone, oocysts continue to be released in the feces for several weeks.

Even though known for a long time, detection of the organism and treatment of the malady it causes are still challenging. No **vaccine** for cryptosporidiosis exists. A well-functioning **immune system** is the best defense against the disease. Indeed, estimates are that about 30% of the population has antibodies to *Cryptosporidium parvum*, even though no symp-

toms of cryptosporidiosis developed. The malady is most severe in immunocompromised people, such as those infected with **HIV** (the virus that causes AIDS), or those receiving **chemotherapy** for cancer or after a transplant. For those who are diabetic, alcoholic, or pregnant, the prolonged diarrhea can be dangerous.

In another avenue of infection, some of the merozoites grow bigger inside the host epithelial cell and form two other types of cells, termed the macrogametocyte and microgametocyte. The macrogametocytes contain macrogametes. When these combine with the microgametes released from the microgametocytes, a zygote is formed. An oocyst wall forms around the zygote and the genetic process of meiosis results in the creation of four sporozoites inside the oocyst. The oocyst is released to the environment in the feces and the infectious cycle is started again.

The cycle from ingestion to the release of new infectious oocytes in the feces can take about four days. Thereafter, the production of a new generation of **parasites** takes as little as twelve to fourteen hours. Internally, this rapid division can create huge numbers of organisms, which crowd the intestinal tract. Cryptosporidiosis can spread to secondary sites, like the duodenum and the large intestine. In people whose immune systems are not functioning properly, the spread of the organism can be even more extensive, with parasites being found in the stomach, biliary tract, pancreatic ducts, and respiratory tract.

Detection of Cryptosporidium in water is complicated by the lack of a **culture** method and because large volumes of water (hundreds of gallons) need to be collected and concentrated to collect the few oocytes that may be present. Presently, oocysts are detected using a microscopic method involving the binding of a specific fluorescent probe to the oocyte wall. There are many other noninfectious species of Cryptosporidium in the environment that react with the probe used in the test. Furthermore, the test does not distinguish a living organism from one that is dead. So a positive test result is not always indicative of the presence of an infectious organism. Skilled analysts are required to perform the test and so the accuracy of detection varies widely from lab to lab.

See also Giardia and giardiasis; Water quality; Water purification

CULTURE

A culture is a single species of microorganism that is isolated and grown under controlled conditions. The German bacteriologist **Robert Koch** first developed culturing techniques in the late 1870s. Following Koch's initial discovery, medical scientists quickly sought to identify other pathogens. Today **bacteria** cultures are used as basic tools in microbiology and medicine.

The ability to separate bacteria is important because **microorganisms** exist as mixed populations. In order to study individual species, it is necessary to first isolate them. This isolation can be accomplished by introducing individual bacterial cells onto a culture medium containing the necessary

Liquid cultures of luminescent bacteria.

elements microbial growth. The medium also provides conditions favorable for growth of the desired species. These conditions may involve **pH**, osmotic pressure, atmospheric oxygen, and moisture content. Culture media may be liquids (known broths) or solids. Before the culture can be grown, the media must be sterilized to prevent growth of unwanted species. This **sterilization** process is typically done through exposure to high temperatures. Some tools like the metal loop used to introduce bacteria to the media, may be sterilized by exposure to a flame. The media itself may be sterilized by treatment with steam-generated heat through a process known as autoclaving.

To grow the culture, a number of the cells of the microorganism must be introduced to the sterilized media. This process is known as inoculation and is typically done by exposing an inoculating loop to the desired strain and then placing the loop in contact with the sterilized surface. A few of the cells will be transferred to the growth media and under the proper conditions, that species will begin to grow and form a pure **colony**. Cells in the colony can reproduce as often as every 20 minutes and under the ideal conditions, this rate of cell division could result in the production of 500,000 new

cells after six hours. Such rapid growth rates help to explain the rapid development of disease, food spoilage, decay, and the speed at which certain chemical processes used in industry take place. Once the culture has been grown, a variety of observation methods can be used to record the strain's characteristics and chart its growth.

See also Agar and agarose; Agar diffusion; American type culture collection; Antibiotic resistance, tests for; Bacterial growth and division; Bacterial kingdoms; Epidemiology, tracking diseases with technology; Laboratory techniques in microbiology

CYCLOSPORIN • *see* ANTIBIOTICS

CYTOGENETICS • *see* MOLECULAR BIOLOGY AND MOLECULAR GENETICS

CYTOKINES

Cytokines are a family of small proteins that mediate an organism's response to injury or infection. Cytokines operate by transmitting signals between cells in an organism. Minute quantities of cytokines are secreted, each by a single cell type, and regulate functions in other cells by binding with specific receptors. Their interactions with the receptors produce secondary signals that inhibit or enhance the action of certain genes within the cell. Unlike endocrine hormones, which can act throughout the body, most cytokines act locally, near the cells that produced them.

Cytokines are crucial to an organism's self-defense. Cells under attack release a class of cytokines known as chemokines. Chemokines participate in a process called chemotaxis, signaling white blood cells to migrate toward the threatened region. Other cytokines induce the white blood cells to produce **inflammation**, emitting toxins to kill pathogens and **enzymes** to digest both the invaders and the injured tissue. If the inflammatory response is not enough to deal with the problem, additional **immune system** cells are also summoned by cytokines to continue the fight.

In a serious injury or infection, cytokines may call the hematopoietic, or blood-forming system into play. New white blood cells are created to augment the immune response, while additional red blood cells replace any that have been lost. Ruptured blood vessels emit chemokines to attract platelets, the element of the blood that fosters clotting. Cytokines are also responsible for signaling the nervous system to increase the organism's metabolic level, bringing on a fever that inhibits the proliferation of pathogens while boosting the action of the immune system.

Because of the central role of cytokines in fighting infection, they are being studied in an effort to find better treatments for diseases such as **AIDS**. Some have shown promise as therapeutic agents, but their usefulness is limited by the tendency of cytokines to act locally. This means that their short amino acid chains are likely either to be destroyed by enzymes in the bloodstream or tissues before reaching their destination, or to act on other cells with unintended consequences.

Other approaches to developing therapies based on research into cytokines involve studying their receptor sites on target cells. If a molecule could be developed that would bind to the receptor site of a specific cytokine, it could elicit the desired action from the cell, and might be more durable in the bloodstream or have other advantages over the native cytokine. Alternatively, a drug that blocked receptor sites could potentially prevent the uncontrolled inflammatory responses seen in certain autoimmune diseases.

See also Autoimmunity and autoimmune diseases; Immunochemistry; Immunodeficiency disease syndromes; Immunodeficiency diseases

CYTOPLASM, EUKARYOTIC

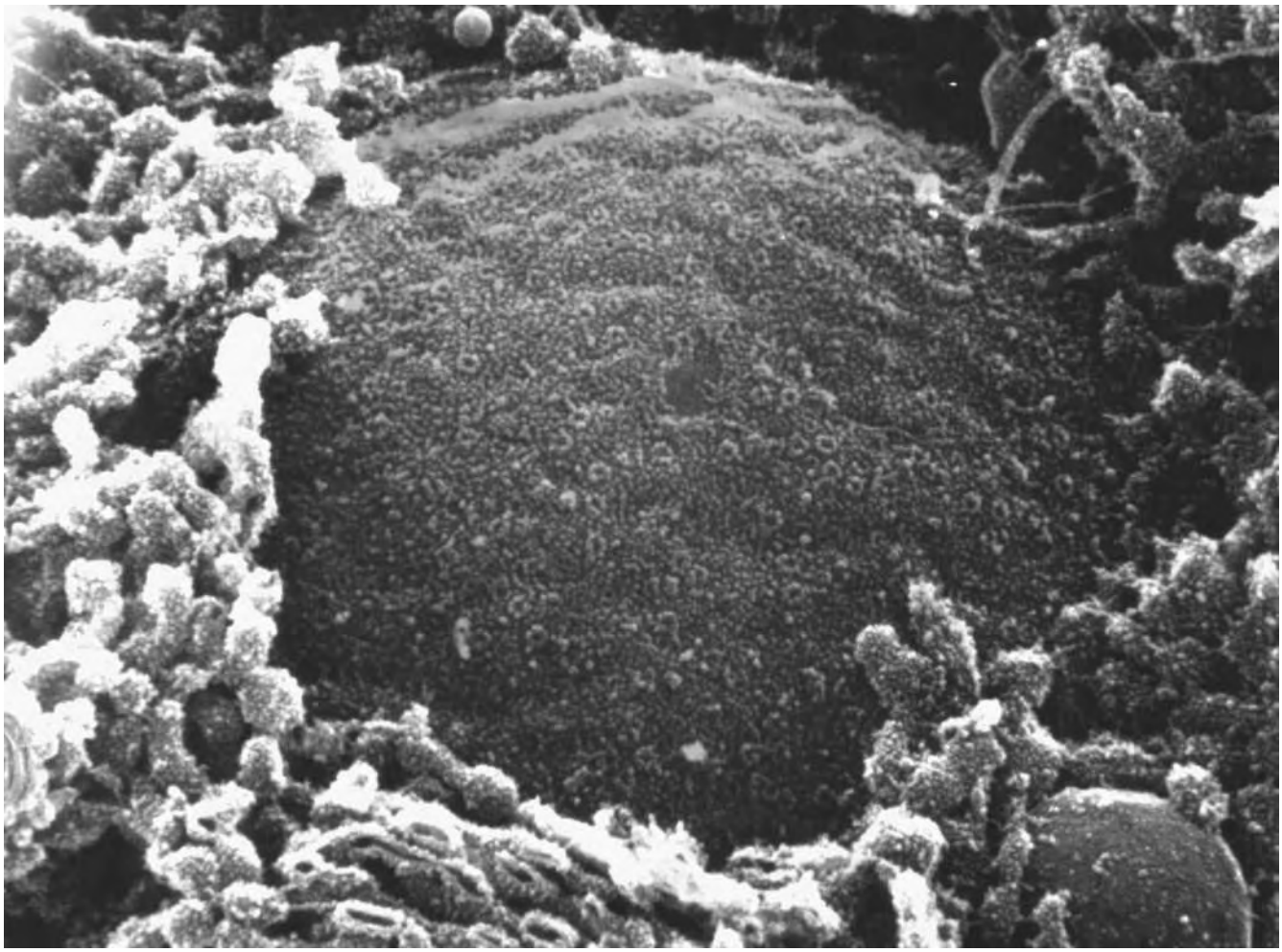
The cytoplasm, or cytosol of eukaryotic cells is the gel-like, water-based fluid that occupies the majority of the volume of the cell. Cytoplasm functions as the site of energy production, storage, and the manufacture of cellular components. The various organelles that are responsible for some of these functions in the eukaryotic cell are dispersed throughout the cytoplasm, as are the compounds that provide structural support for the cell.

The cytoplasm is the site of almost all of the chemical activity occurring in a eukaryotic cell. Indeed, the word cytoplasm means "cell substance."

Despite being comprised mainly of water (about 65% by volume), the cytoplasm has the consistency of gelatin. Unlike gelatin, however, the cytoplasm will flow. This enables **eukaryotes** such as the amoeba to adopt different shapes, and makes possible the formation of pseudopods that are used to engulf food particles. The consistency of the cytoplasm is the result of the other constituents of the cell that are floating in fluid. These constituents include salts, and organic molecules such as the many **enzymes** that catalyze the myriad of chemical reactions that occur in the cell.

When viewed using the transmission electron **microscope**, the cytoplasm appears as a three-dimensional latticework of strands. In the early days of **electron microscopy** there was doubt as to whether this appearance reflected the true nature of the cytoplasm, or was an artifact of the removal of water from the cytoplasm during the preparation steps prior to **electron microscopic examination**. However, development of techniques that do not perturb the natural structure biological specimens has confirmed that this latticework is real.

The lattice is made of various cytoplasmic proteins. They are scaffolding structures that assist in the process of cell division and in the shape of the cell. The shape-determinant is referred to as the cytoskeleton. It is a network of fibers composed of three types of proteins. The proteins form three filamentous structures known as microtubules, intermediate filaments, and microfilaments. The filaments are connected to most of organelles located in the cytoplasm and serve to hold together the organelles.



Scanning electron micrograph of an eukaryotic cell, showing the nucleus in the center surrounded by the cytoplasm. The oval objects to the lower left are ribosomes.

The microtubules are tubes that are formed by a spiral arrangement of the constituent protein. They function in the movement of the **chromosomes** to either pole of the cell during the cell division process. The microtubules are also known as the spindle apparatus. Microfilaments are a composed of two strands of protein that are twisted around one another. They function in the contraction of muscle in higher eukaryotic cells and in the change in cell shape that occurs in organisms such as the amoeba. Finally, the intermediate filaments act as more rigid scaffolding to maintain the cell shape.

The organelles of the cell are dispersed throughout the cytoplasm. The **nucleus** is bound by its own membrane to protect the genetic material from potentially damaging reactions that occur in the cytoplasm. Thus, the cytoplasm is not a part of the interior of the organelles.

The cytoplasm also contains **ribosomes**, which float around and allow protein to be synthesized all through the cell. Ribosomes are also associated with a structure called the endoplasmic reticulum. The golgi apparatus is also present, in association with the endoplasmic reticulum. Enzymes that degrade compounds are in the cytoplasm, in organelles called lysosomes. Also present throughout the cytoplasm are the mitochondria, which are the principal energy generating structures of the cell. If the eukaryotic cell is capable of photosynthetic activity, then **chlorophyll** containing organelles known as chloroplasts are also present.

The cytoplasm of eukaryotic cells also functions to transport dissolved nutrients around the cell and move waste material out of the cell. These functions are possible because of a process dubbed cytoplasmic streaming.

See also Eukaryotes

CYTOPLASM, PROKARYOTIC

The cytoplasm of a prokaryotic cell is everything that is present inside the bacterium. In contrast to a eukaryotic cell, there is not a functional segregation inside **bacteria**. The cytoplasm houses all the chemicals and components that are used to sus-

tain the life of a bacterium, with the exception of those components that reside in the membrane(s), and in the **periplasm** of Gram-negative bacteria.

The cytoplasm is bounded by the cytoplasmic membrane. Gram-negative bacteria contain another outer membrane. In between the two membranes lies the periplasm.

When viewed in the light **microscope**, the cytoplasm of bacteria is transparent. Only with the higher magnification available using the transmission **electron microscope** does the granular nature of the cytoplasm become apparent. The exact structure of the cytoplasm may well be different than this view, since the cytoplasm is comprised mainly of water. The dehydration necessary for conventional electron microscopy likely affect the structure of the cytoplasm.

The cytoplasm of prokaryotes and **eukaryotes** is similar in texture. Rather than being a free-flowing liquid the cytoplasm is more of a gel. The consistency has been likened to that of dessert gel, except that the bacterial gel is capable of flow. The ability of flow is vital, since the molecules that reside in the cytoplasm must be capable of movement within the bacterium as well as into and out of the cytoplasm.

The genetic material of the bacteria is dispersed throughout the cytoplasm. Sometimes, the **deoxyribonucleic acid** genome can aggregate during preparation for microscopy. Then, the genome is apparent as a more diffuse area within the granular cytoplasm. This artificial structure has been called the nucleoid. Smaller, circular arrangements of genetic material called **plasmids** can also be present. The dispersion of the bacterial genome throughout the cytoplasm is one of the fundamental distinguishing features between prokaryotic and eukaryotic cells.

Also present throughout the cytoplasm is the **ribonucleic acid**, various **enzymes**, amino acids, carbohydrates, lipids, ions, and other compounds that function in the bacterium. The constituents of the membrane(s) are manufactured in the cytoplasm and then are transported to their final destination.

Some bacteria contain specialized regions known as cytoplasmic inclusions that perform specialized functions. These inclusions can be stored products that are used for the nutrition of the bacteria. Examples of such inclusions are glycogen, poly-B-hydroxybutyrate, and sulfur granules. As well, certain bacteria contain gas-filled vesicles that act to buoy the bacterium up to a certain depth in the water, or membranous structures that contain **chlorophyll**. The latter function to harvest light for energy in photosynthetic bacteria.

The cytoplasm of prokaryotic cells also houses the **ribosomes** required for the manufacture of protein. There can be many ribosomes in the cytoplasm. For example, a rapidly growing bacterium can contain upwards of 15,000 ribosomes.

The processes of **transcription**, **translation**, protein import and export, and at least some degradation of compounds occurs in the cytoplasm. In Gram-negative bacteria, some of these functions also occur in the periplasmic fluid. The mechanisms that underlie the proper sequential orchestration of these functions are still yet to be fully determined.

See also Bacterial ultrastructure

D

D'HÉRELLE, FÉLIX (1873-1949)

Canadian bacteriologist

Félix d'Hérelle's major contribution to science was the discovery of the **bacteriophage**, a microscopic agent that appears in conjunction with and destroys disease-producing **bacteria** in a living organism. Like many researchers, d'Hérelle spent much of his life exploring the effects of his major discovery. He was also well-traveled; in the course of his life he lived for long or short periods of time in Canada, France, the Netherlands, Guatemala, Mexico, Indochina, Egypt, India, the United States, and the former Soviet Union.

D'Hérelle was born in Montreal, Quebec, Canada. His father, Félix d'Hérelle—a member of a well-established French Canadian family, died when the young Félix was six years old. After his father's death, he moved with his mother, Augustine Meert d'Hérelle, a Dutch woman, to Paris, France. In Paris, d'Hérelle received his secondary education at the Lycée Louis-le-Grand and began his medical studies. He completed his medical program at the University of Leiden in the Netherlands. He married Mary Kerr, of France, in 1893, and the couple eventually had two daughters. In 1901, d'Hérelle moved to Guatemala City, Guatemala, to become the director of the bacteriology laboratory at the general hospital and to teach microbiology at the local medical school. In 1907, he moved to Merida, Yucatan, Mexico, to study the **fermentation** of sisal hemp, and in 1908, the Mexican government sent him back to Paris to further his microbiological studies. D'Hérelle became an assistant at Paris's Pasteur Institute in 1909, became chief of its laboratory in 1914, and remained at the Institute until 1921.

During his time at the Pasteur Institute, d'Hérelle studied a bacterium called *Coccobacillus acridiorum,* which caused enteritis (**inflammation** of the intestines) in locusts and grasshoppers of the acrididae family of insects, with a view toward using the microbe to destroy locusts. In growing the bacteria on **culture** plates, d'Hérelle observed empty spots on the plates and theorized that these spots resulted from a virus that grew along with and killed the bacteria. He surmised that this phenomenon might have great medical significance as an example of an organism fighting diseases of the digestive tract. In 1916, he extended his investigation to cultures of the bacillus that caused **dysentery** and again observed spots free of the microbe on the surface of the cultures. He was able to filter out a substance from the feces of dysentery victims that consumed in a few hours a culture broth of the bacillus. On September 10, 1917, he presented to the French Academy of Sciences a paper announcing his discovery entitled "Sur un microbe invisible, antagoniste du bacille dysentérique." He named the bacteria–destroying substance bacteriophage (literally, "eater of bacteria"). He devoted most of his research and writing for the rest of his life to the various types of bacteriophage which appeared in conjunction with specific types of bacteria. He published several books dealing with his findings.

From 1920 to the late 1930s, d'Hérelle traveled and lived in many parts of the world. In 1920, he went to French Indochina under the auspices of the Pasteur Institute to study human dysentery and septic pleuropneumonia in buffaloes. It was during the course of this expedition that he perfected his techniques for isolating bacteriophage. From 1922 to 1923, he served as an assistant professor at the University of Leiden. In 1924, he moved to Alexandria, Egypt, to direct the Bacteriological Service of the Egyptian Council on Health and Quarantine. In 1927, he went to India at the invitation of the Indian Medical Service to attempt to cure cholera through the use of the bacteriophage associated with that disease. D'Hérelle served as professor of bacteriology at Yale University from 1928 to 1933, and in 1935 the government of the Soviet Socialist Republic of Georgia requested that d'Hérelle establish institutes dedicated to the study of bacteriophage in Tiflis, Kiev, and Kharkov. However, unstable civil conditions forced d'Hérelle's departure from the Soviet Union in 1937, and he returned to Paris, where he lived, continuing his study of bacteriophage, for the remainder of his life.

D'Hérelle attempted to make use of bacteriophage in the treatment of many human and animal diseases, including

dysentery, cholera, plague, and staphylococcus and streptococcus infections. Such treatment was widespread for a time, especially in the Soviet Union. However, use of bacteriophage for this purpose was superseded by the use of chemical drugs and **antibiotics** even within d'Hérelle's lifetime. Today bacteriophage is employed primarily as a diagnostic ultravirus. Of the many honors d'Hérelle received, his perhaps most notable is the Leeuwenhoek Medal given to him by the Amsterdam Academy of Science in 1925; before d'Hérelle, **Louis Pasteur** had been the only other French scientist to receive the award. D'Hérelle was presented with honorary degrees from the University of Leiden and from Yale, Montreal, and Laval Universities. He died after surgery in Paris at the age of 75.

See also Bacteriophage and bacteriophage typing

DARWIN, CHARLES ROBERT (1809-1882)

English naturalist

Charles Robert Darwin is credited with popularizing the concept of organic **evolution** by means of natural **selection**. Though Darwin was not the first naturalist to propose a model of biological evolution, his introduction of the mechanism of the "survival of the fittest," and discussion of the evolution of humans, marked a revolution in both science and natural philosophy.

Darwin was born in Shrewsbury, England and showed an early interest in the natural sciences, especially geology. His father, Robert Darwin, a wealthy physician, encouraged Charles to pursue studies in medicine at the University of Edinburg. Darwin soon tired of the subject, and his father sent him to Cambridge to prepare for a career in the clergy. At Cambridge, Darwin rekindled his passion for the natural sciences, often devoting more time to socializing with Cambridge scientists than to his clerical studies. With guidance from his cousin, entomologist William Darwin Fox (1805–1880), Darwin became increasingly involved in the growing circle of natural scientists at Cambridge. ox introduced Darwin to clergyman and biologist John Stevens Henslow (1796–1861). Henslow became Darwin's tutor in mathematics and theology, as well as his mentor in his personal studies of botany, geology, and zoology. Henslow profoundly influenced Darwin, and it was he who encouraged Darwin to delay seeking an appointment in the Church of England in favor of joining an expedition team and venturing overseas. After graduation, Darwin agreed to an unpaid position as naturalist aboard the *H.M.S. Beagle*. The expedition team was initially chartered for a three year voyage and survey of South America's Pacific coastline, but the ship pursued other ventures after their work was complete and Darwin remained part of H.M.S. *Beagle*'s crew for five years.

Darwin used his years aboard the *Beagle* to further his study of the natural sciences. In South America, Darwin became fascinated with geology. He paid close attention to changes in the land brought about by earthquakes and volcanoes. His observations led him to reject catastrophism (a theory that land forms are the result of single, catastrophic events), and instead espoused the geological theories of gradual development proposed by English geologist Charles Lyell (1797–1875) in his 1830 work, *Principles of Geology*. Yet, some of his observations in South America did not fit with Lyell's theories. Darwin disagreed with Lyell's assertion that coral reefs grew atop oceanic volcanoes and rises, and concluded that coral reefs built upon themselves. When Darwin returned to England in 1836, he and Lyell became good friends. Lyell welcomed Darwin's new research on coral reefs, and encouraged him to publish other studies from his voyages.

Darwin was elected a fellow of the Geological Society in 1836, and became a member of the Royal Society in 1839. That same year, he published his *Journal of Researches into the Geology and Natural History of the Various Countries Visited by H.M.S. Beagle*. Though his achievements in geology largely prompted his welcoming into Britain's scientific community, his research interests began to diverge from the discipline in the early 1840s. Discussions with other naturalists prompted Darwin's increasing interest in population diversity of fauna, extinct animals, and the presumed fixity of species. Again, he turned to notes of his observations and various specimens he gathered while on his prior expedition. The focus of his new studies was the Galápagos Islands off the Pacific coast of Ecuador. While there, Darwin was struck by the uniqueness of the island's tortoises and birds. Some neighboring islands had animal populations, which were largely similar to that of the continent, while others had seemingly different variety of species. After analyzing finch specimen from the Galápagos, Darwin concluded that species must have some means of transmutation, or ability of a species to alter over time. Darwin thus proposed that as species modified, and as old species disappeared, new varieties could be introduced. Thus, Darwin proposed an evolutionary model of animal populations.

The idea of organic evolution was not novel. French naturalist, Georges Buffon (1707–1788) had theorized that species were prone to development and change. Darwin's own grandfather, Erasmus Darwin, also published research regarding the evolution of species. Although the theoretical concept of evolution was not new, it remained undeveloped prior to Charles Darwin. Just as he had done with Lyell's geological theory, Darwin set about the further the understanding of evolution not merely as a philosophical concept, but as a practical scientific model for explaining the diversity of species and populations. His major contribution to the field was the introduction of a mechanism by which evolution was accomplished. Darwin believed that evolution was the product of an ongoing struggle of species to better adapt to their environment, with those that were best adapted surviving to reproduce and replace less-suited individuals. He called this phenomenon "survival of the fittest," or natural selection. In this way, Darwin believed that traits of maximum adaptiveness were transferred to future generations of the animal population, eventually resulting in new species.

Darwin finished an extensive draft of his theories in 1844, but lacked confidence in his abilities to convince others of the merits of his discoveries. Years later, prompted by rumors that a colleague was about to publish a theory similar

to his own, Darwin decided to release his research. *On the Origin of Species by Means of Natural Selection, or The Preservation of Favoured Races in the Struggle for Life*, was published November 1859, and became an instant bestseller.

A common misconception is that *On the Origin of Species* was the introduction of the concept of human evolution. In fact, a discussion of human antiquity is relatively absent from the book. Darwin did not directly address the relationship between animal and human evolution until he published *The Descent of Man, and Selection in Relation to Sex* in 1871. Darwin introduced not only a model for the biological evolution of man, but also attempted to chart the process of man's psychological evolution. He further tried to break down the barriers between man and animals in 1872 with his work *The Expression of the Emotions in Man and Animals*. By observing facial features and voice sounds, Darwin asserted that man and non-human animals exhibited signs of emotion in similar ways. In the last years of his career, Darwin took the concept of organic evolution to its logical end by applying natural selection and specialization to the plant kingdom.

Darwin's works on evolution met with both debate from the scientific societies, and criticism from some members of the clergy. *On the Origin of Species* and *The Descent of Man* were both published at a time of heightened religious evangelicalism in England. Though willing to discuss his theories with colleagues in the sciences, Darwin refrained from participating in public debates concerning his research. In the last decade of his life, Darwin was disturbed about the application of his evolutionary models to social theory. By most accounts, he considered the emerging concept of the social and cultural evolution of men and civilizations, which later became known as Social Darwinism, to be a grievous misinterpretation of his works. Regardless of his opposition, he remained publicly taciturn about the impact his scientific theories on theology, scientific methodology, and social theory. Closely guarding his privacy, Darwin retired to his estate in Down. He died at Down House in 1882. Though his wishes were to receive an informal burial, Parliament immediately ordered a state burial for the famous naturalist at Westminster Abby. By the time of his death, the scientific community had largely accepted the arguments favoring his theories of evolution. Although the later discoveries in genetics and **molecular biology** radically reinterpreted Darwin's evolutionary mechanisms, evolutionary theory is the key and unifying theory in all biological science.

See also Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses

DAVIES, JULIAN E. (1932-)

Welsh bacteriologist

Julian Davies is a bacteriologist renowned for his research concerning the mechanisms of bacterial resistance to **antibiotics**, and on the use of antibiotics as research tools.

Davies was born in Casrell Nedd, Morgannwg, Cymru, Wales. He received his education in Britain. His university education was at the University of Nottingham, where he received a B.Sc. (Chemistry, Physics, Math) in 1953 and a Ph.D. (Organic Chemistry) in 1956. From 1959 to 1962, he was Lecturer at the University of Manchester. Davies then moved to the United States where he was an Associate at the Harvard Medical School from 1962 until 1967. From 1965 to 1967, he was also a Visiting Professor at the Institute Pasteur in Paris. In 1967, Davies became an Associate Professor in the Department of **Biochemistry** at the University of Wisconsin. He attained the rank of Professor in 1970 and remained at Wisconsin until 1980. In that year, Davies took up the post of Research Director at Biogen in Geneva. In 1983, he became President of Biogen. Two years later, Davies assumed the position of Chief of Genetic Microbiology at the Institute Pasteur, where he remained until 1992. In that year, he returned to North America to become Professor and Head of the Department of Microbiology and **Immunology** at UBC. He retained this position until his retirement in 1997. Presently he remains affiliated with UBC as Emeritus Professor in the same department.

While in British Columbia, Davies returned to commercial **biotechnology**. In 1996, he founded and became President and CEO of TerraGen Diversity Inc. Davies assumed the post of Chief Scientific Officer from 1998 to 2000. From 2000 to the present, he is Executive Vice President, technology development of Cubist Pharmaceuticals, Inc.

Davies has made fundamental discoveries in the area of bacterial **antibiotic resistance**, including the origin and **evolution** of antibiotic resistance genes. He has identified bacterial **plasmids** that carry genes that carry the information that determines the resistance of **bacteria** to certain antibiotics. Furthermore, he demonstrated that this information could be transferred from one bacterium to another. These discoveries have crucial to the efforts to develop drugs that can overcome such antibiotic resistance.

Another facet of research has demonstrated how genetic information can be transferred between bacteria that are distantly related. This work has had a fundamental influence on the understanding of how bacteria can acquire genetic traits, especially those that lead to antimicrobial resistance.

Davies has also developed a technique whereby genes can be "tagged" and their path from one bacterium to another followed. This technique is now widely used to follow **gene** transfer between prokaryotic and eukaryotic cells. In another research area, Davies has explored the use of antibiotics as experimental tools to probe the mechanisms of cellular biochemistry, and the interaction between various molecules in cells.

This prodigious research output has resulted in over 200 publications in peer-reviewed journals, authorship of six books and numerous guest lectures.

Davies has also been active as an undergraduate and graduate teacher and a mentor to a number of graduate students. These research, commercial and teaching accomplishments have been recognized around the world. He is a Fellow of the Royal Society (London) and the Royal Society of Canada, and is a past President of the American Society for Microbiology. In 2000, he received a lifetime achievement

award in recognition of his development of the biotechnology sector in British Columbia.

See also Microbial genetics

BROGLIE, LOUIS VICTOR DE (1892-1987)

French physicist

Louis Victor de Broglie, a theoretical physicist and member of the French nobility, is best known as the father of wave mechanics, a far-reaching achievement that significantly changed modern physics. Wave mechanics describes the behavior of matter, including subatomic particles such as electrons, with respect to their wave characteristics. For this groundbreaking work, de Broglie was awarded the 1929 Nobel Prize for physics. De Broglie's work contributed to the fledgling science of microbiology in the mid-1920s, when he suggested that electrons, as well as other particles, should exhibit wave-like properties similar to light. Experiments on electron beams a few years later confirmed de Broglie's hypothesis. Of importance to **microscope** design was the fact that the wavelength of electrons is typically much smaller than the wavelength of light. Therefore, the limitation imposed on the light microscope of 0.4 micrometers could be significantly reduced by using a beam of electrons to illuminate the specimen. This fact was exploited in the 1930s in the development of the **electron microscope**.

Louis Victor Pierre Raymond de Broglie was born on August 15, 1892, in Dieppe, France, to Duc Victor and Pauline d'Armaille Broglie. His father's family was of noble Piedmontese origin and had served French monarchs for centuries, for which it was awarded the hereditary title Duc from King Louis XIV in 1740, a title that could be held only by the head of the family.

The youngest of five children, de Broglie inherited a familial distinction for formidable scholarship. His early education was obtained at home, as befitted a great French family of the time. After the death of his father when de Broglie was fourteen, his eldest brother Maurice arranged for him to obtain his secondary education at the Lycée Janson de Sailly in Paris.

After graduating from the Sorbonne in 1909 with baccalaureates in philosophy and mathematics, de Broglie entered the University of Paris. He studied ancient history, paleography, and law before finding his niche in science, influenced by the writings of French theoretical physicist Jules Henri Poincaré. The work of his brother Maurice, who was then engaged in important, independent experimental research in x rays and radioactivity, also helped to spark de Broglie's interest in theoretical physics, particularly in basic atomic theory. In 1913, he obtained his Licencié ès Sciences from the University of Paris's Faculté des Sciences.

De Broglie's studies were interrupted by the outbreak of World War I, during which he served in the French army. Yet, even the war did not take the young scientist away from the country where he would spend his entire life; for its duration, de Broglie served with the French Engineers at the wireless station under the Eiffel Tower. In 1919, de Broglie returned to his scientific studies at his brother's laboratory. Here he began his investigations into the nature of matter, inspired by a conundrum that had long been troubling the scientific community: the apparent physical irreconcilability of the experimentally proven dual nature of light. Radiant energy or light had been demonstrated to exhibit properties associated with particles as well as their well-documented wave-like characteristics. De Broglie was inspired to consider whether matter might not also exhibit dual properties. In his brother's laboratory, where the study of very high frequency radiation using spectroscopes was underway, de Broglie was able to bring the problem into sharper focus. In 1924, de Broglie, with over two dozen research papers on electrons, atomic structure, and x rays already to his credit, presented his conclusions in his doctoral thesis at the Sorbonne. Entitled "Investigations into the Quantum Theory," it consolidated three shorter papers he had published the previous year.

In his thesis, de Broglie postulated that all matter—including electrons, the negatively charged particles that orbit an atom's **nucleus**—behaves as both a particle and a wave. Wave characteristics, however, are detectable only at the atomic level, whereas the classical, ballistic properties of matter are apparent at larger scales. Therefore, rather than the wave and particle characteristics of light and matter being at odds with one another, de Broglie postulated that they were essentially the same behavior observed from different perspectives. Wave mechanics could then explain the behavior of all matter, even at the atomic scale, whereas classical Newtonian mechanics, which continued to accurately account for the behavior of observable matter, merely described a special, general case. Although, according to de Broglie, all objects have "matter waves," these waves are so small in relation to large objects that their effects are not observable and no departure from classical physics is detected. At the atomic level, however, matter waves are relatively larger and their effects become more obvious. De Broglie devised a mathematical formula, the matter wave relation, to summarize his findings.

American physicist Albert Einstein appreciated the significant of de Broglie's theory; de Broglie sent Einstein a copy of his thesis on the advice of his professors at the Sorbonne, who believed themselves not fully qualified to judge it. Einstein immediately pronounced that de Broglie had illuminated one of the secrets of the Universe. Austrian physicist Erwin Schrödinger also grasped the implications of de Broglie's work and used it to develop his own theory of wave mechanics, which has since become the foundation of modern physics.

De Broglie's wave matter theory remained unproven until two separate experiments conclusively demonstrated the wave properties of electrons—their ability to diffract or bend, for example. American physicists Clinton Davisson and Lester Germer and English physicist George Paget Thomson all proved that de Broglie had been correct. Later experiments would demonstrate that de Broglie's theory also explained the behavior of protons, atoms, and even molecules. These properties later found practical applications in the development of magnetic lenses, the basis for the electron microscope.

In 1928, de Broglie was appointed professor of theoretical physics at the University of Paris's Faculty of Science. De Broglie was a thorough lecturer who addressed all aspects of wave mechanics. Perhaps because he was not inclined to encourage an interactive atmosphere in his lectures, he had no noted record of guiding young research students.

During his long career, de Broglie published over twenty books and numerous research papers. His preoccupation with the practical side of physics is demonstrated in his works dealing with cybernetics, atomic energy, particle accelerators, and wave-guides. His writings also include works on x rays, gamma rays, atomic particles, optics, and a history of the development of contemporary physics. He served as honorary president of the French Association of Science Writers and, in 1952, was awarded first prize for excellence in science writing by the Kalinga Foundation. In 1953, Broglie was elected to London's Royal Society as a foreign member and, in 1958, to the French Academy of Arts and Sciences in recognition of his formidable output. With the death of his older brother Maurice two years later, de Broglie inherited the joint titles of French duke and German prince. De Broglie died of natural causes on March 19, 1987, at the age of ninety-four.

See also Electron microscope, transmission and scanning; Electron microscopic examination of microorganisms; Microscope and microscopy

DEFECTS OF CELLULAR IMMUNITY • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

DEFECTS OF T CELL MEDIATED IMMUNITY • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

DENGUE FEVER

Dengue fever is a debilitating and sometimes hemorrhagic fever (one that is associated with extensive internal bleeding). The disease is caused by four slightly different types of a virus from the genus *Flavivirus* that is designated as DEN. The four antigenic types are DEN-1, DEN-2, DEN-3, and DEN-4.

The dengue virus is transmitted to humans via the bite of a mosquito. The principle mosquito species is known as *Aedes aegypti*. This mosquito is found all over the world, and, throughout time, became adapted to urban environments. For example, the mosquito evolved so as to be capable of living year round in moist storage containers, rather than relying on the seasonal patterns of rainfall. Another species, *Aedes albopictus* (the "Tiger mosquito"), is widespread throughout Asia. Both mosquitoes are now well established in urban centers. Accordingly, dengue fever is now a disease of urbanized, developed areas, rather than rural, unpopulated regions.

The dengue virus is passed to humans exclusively by the bite of mosquito in search of a blood meal. This mode of transmission makes the dengue virus an arbovirus (that is, one that is transmitted by an arthropod). Studies have demonstrated that some species of monkey can harbor the virus. Thus, monkeys may serve as a reservoir of the virus. Mosquitoes who bite the monkey may acquire the virus and subsequently transfer the virus to humans.

The disease has been known for centuries. The first reported cases were in 1779–1780, occurring almost simultaneously in Asia, Africa, and North America. Since then, periodic outbreaks of the disease have occurred in all areas of the world where the mosquito resides. In particular, an outbreak that began in Asia after World War II, spread around the world, and has continued to plague southeast Asia even into 2002. As of 2001, dengue fever was the leading cause of hospitalization and death among children in southeast Asia.

Beginning in the 1980s, dengue fever began to increase in the Far East and Africa. Outbreaks were not related to economic conditions. For example, Singapore had an outbreak of dengue fever from 1990 to 1994, even after a mosquito control program that had kept the disease at minimal levels for over two decades. The example of Singapore illustrates the importance of an ongoing program of mosquito population control.

The disease is a serious problem in more than 100 countries in Africa, North and South America, the Eastern Mediterranean, South-East Asia, and the Western Pacific.

Unlike other bacterial or viral diseases, which can be controlled by **vaccination**, the four antigenic types of the dengue virus do not confer cross-protection. Thus, it is possible for an individual to be sickened with four separate bouts of dengue fever.

Following the transfer of the virus from mosquito to humans, the symptoms can be varied, ranging from nonspecific and relatively inconsequential ailments to severe and fatal hemorrhaging. The incubation period of the virus is typically 5 to 8 days, but symptoms may develop after as few as three days or as many as 15 days. The onset of symptoms is sudden and dramatic. Initially, chills tend to develop, followed by a headache. Pain with the movement of the eyes leads to more generalized and extreme pain in the legs and joints. A high fever can be produced, with temperatures reaching 104° F [40° C]. Also, a pale rash may appear transiently on the face.

These symptoms can persist for up to 96 hours. Often, the fever then rapidly eases. After a short period when symptoms disappear, the fever reappears. The temperature elevates rapidly but the fever is usually not as high as in previous episodes. The palms of the hands and soles of the feet may turn bright red and become very swollen.

In about 80% of those who are infected, recovery is complete after a convalescent period of several weeks with general weakness and lack of energy. However, in some 20% of those who are infected a severe form of dengue fever develops. This malady is characterized by the increased leakage of fluid from cells and by the abnormal clotting of the blood. These factors produce the hemorrhaging that can be a hallmark of the disease, which is called dengue hemorrhagic fever. Even then, recovery can be complete within a week. Finally, in some of those who are infected, a condition called dengue shock syndrome can result in convulsions. In addition, a failure of the circulatory system can occur, resulting in death.

The reasons for the varied degrees of severity and symptoms that the viral infection can elicit are still unclear. Not surprisingly, there is currently no cure for dengue, nor is there a **vaccine**. Treatment for those who are afflicted is palliative, that is, intended to ease the symptoms of the disease. Upon recovery, **immunity** to the particular antigenic type of the virus is in place for life. However, an infection with one antigenic type of dengue virus is not protective against the other three antigenic types. Currently, the only preventive measure that can be taken is to eradicate the mosquito vector of the virus.

See also Epidemics, viral; Zoonoses

DEOXYRIBONUCLEIC ACID • *see* DNA (DEOXYRIBONUCLEIC ACID)

DESICCATION

Desiccation is the removal of water from a biological system. Usually this is accomplished by exposure to dry heat. Most biological systems are adversely affected by the loss of water. **Microorganisms** are no exception to this, except for those that have evolved defensive measures to escape the loss of viability typically associated with water loss.

Desiccation also results from the freezing of water, such as in the polar regions on Earth. Water is present at these regions, but is unavailable.

Microorganisms depend on water for their structure and function. Cell membranes are organized with the water-loving portions of the membrane lipids positioned towards the exterior and the water-hating portions pointing inward. The loss of water can throw this structure into disarray. Furthermore, the interior of microorganisms such as **bacteria** is almost entirely comprised of water. Extremely rapid freezing of the water can be a useful means of preserving bacteria and other microorganisms. However, the gradual loss of water will produce lethal changes in the chemistry of the interior **cytoplasm** of cells, collapse of the interior structure, and an alteration in the three-dimensional structure of **enzymes**. These drastic changes caused by desiccation are irreversible.

In the laboratory, desiccation techniques are used to help ensure that glassware is free of viable microbes. Typically, the glassware is placed in a large dry-heat oven and heated at 160° to 170° C [320° to 338° F] for up to two hours. The effectiveness of **sterilization** depends on the penetration of heat into a biological sample.

Some microorganisms have evolved means of coping with desiccation. The formation of a spore by bacteria such as Bacillus and Clostridium allows the genetic material to survive the removal of water. Cysts produced by some protozoans can also resist the destruction of desiccation for long periods of time. Bacterial biofilms might not be totally dehydrated if they are thick enough. Bacteria buried deep within the biofilm might still be capable of growth.

The fact that some microbes on Earth can resist desiccation and then resuscitate when moisture becomes available holds out the possibility of life on other bodies in our solar system, particularly Mars. The snow at the poles of Mars is proof that water is present. If liquid water becomes transiently available, then similar resuscitation of dormant Martian microorganisms could likewise occur.

See also Cryoprotection

DETECTION OF MUTANTS • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

DIATOMS

Algae are a diverse group of simple, nucleated, plant-like aquatic organisms that are primary producers. Primary producers are able to utilize **photosynthesis** to create organic molecules from sunlight, water, and carbon dioxide. Ecologically vital, algae account for roughly half of photosynthetic production of organic material on Earth in both freshwater and marine environments. Algae exist either as single cells or as multicellular organizations. Diatoms are microscopic, single-celled algae that have intricate glass-like outer cell walls partially composed of silicon. Different species of diatom can be identified based upon the structure of these walls. Many diatom species are planktonic, suspended in the water column moving at the mercy of water currents. Others remain attached to submerged surfaces. One bucketful of water may contain millions of diatoms. Their abundance makes them important food sources in aquatic ecosystems. When diatoms die, their cell walls are left behind and sink to the bottom of bodies of water. Massive accumulations of diatom-rich sediments compact and solidify over long periods of time to form rock rich in fossilized diatoms that is mined for use in abrasives and filters.

Diatoms belong to the taxonomic phylum Bacillariophyta. There are approximately 10,000 known diatom species. Of all algae phyla, diatom species are the most numerous. The diatoms are single-celled, eukaryotic organisms, having genetic information sequestered into subcellular compartments called nuclei. This characteristic distinguishes the group from other single-celled photosynthetic aquatic organisms, like the **blue-green algae** that do not possess nuclei and are more closely related to **bacteria**. Diatoms also are distinct because they secrete complex outer cell walls, sometimes called skeletons. The skeleton of a diatom is properly referred to as a frustule.

Diatom frustules are composed of pure hydrated silica within a layer of organic, carbon containing material. Frustules are really comprised of two parts: an upper and lower frustule. The larger upper portion of the frustule is called the epitheca. The smaller lower piece is the hypotheca. The epitheca fits over the hypotheca as the lid fits over a shoe-

box. The singular algal diatom cell lives protected inside the frustule halves like a pair of shoes snuggled within a shoebox.

Frustules are ornate, having intricate designs delineated by patterns of holes or pores. The pores that perforate the frustules allow gases, nutrients, and metabolic waste products to be exchanged between the watery environment and the algal cell. The frustules themselves may exhibit bilateral symmetry or radial symmetry. Bilaterally symmetric diatoms are like human beings, having a single plane through which halves are mirror images of one another. Bilaterally symmetric diatoms are elongated. Radially symmetric diatom frustules have many mirror image planes. No matter which diameter is used to divide the cell into two halves, each half is a mirror image of the other. The combination of symmetry and perforation patterns of diatom frustules make them beautiful biological structures that also are useful in identifying different species. Because they are composed of silica, an inert material, diatom frustules remain well preserved over vast periods of time within geologic sediments.

Diatom frustules found in sedimentary rock are microfossils. Because they are so easily preserved, diatoms have an extensive fossil record. Specimens of diatom algae extend back to the Cretaceous Period, over 135 million years ago. Some kinds of rock are formed nearly entirely of fossilized diatom frustules. Considering the fact that they are microscopic organisms, the sheer numbers of diatoms required to produce rock of any thickness is staggering. Rock that has rich concentrations of diatom fossils is known as diatomaceous earth, or diatomite. Diatomaceous earth, existing today as large deposits of chalky white material, is mined for commercial use in abrasives and in filters. The fine abrasive quality of diatomite is useful in cleansers, like bathtub scrubbing powder. Also, many toothpaste products contain fossil diatoms. The fine porosity of frustules also makes refined diatomaceous earth useful in fine water filters, acting like microscopic sieves that catch very tiny particles suspended in solution.

Fossilized diatom collections also tell scientists a lot about the environmental conditions of past eras. It is known that diatom deposits can occur in layers that correspond to environmental cycles. Certain conditions favor mass deaths of diatoms. Over many years, changes in diatom deposition rates in sediments, then, are preserved as diatomite, providing clues about prehistoric climates.

Diatom cells within frustules contain chloroplasts, the organelles in which photosynthesis occurs. Chloroplasts contain **chlorophyll**, the pigment molecule that allows plants and other photosynthetic organisms to capture solar energy and convert it into usable chemical energy in the form of simple sugars. Because of this, and because they are extremely abundant occupants of freshwater and saltwater habitats, diatoms are among the most important **microorganisms** on Earth. Some estimates calculate diatoms as contributing 20–25% of all carbon fixation on Earth. Carbon fixation is a term describing the photosynthetic process of removing atmospheric carbon in the form of carbon dioxide and converting it to organic carbon in the form of sugar. Due to this, diatoms are essential components of aquatic food chains. They are a major food source for many microorganisms, aquatic animal larvae, and grazing animals like mollusks (snails). Diatoms are even found living on land. Some species can be found in moist soil or on mosses. Contributing to the abundance of diatoms is their primary mode of reproduction, simple asexual cell division. Diatoms divide asexually by mitosis. During division, diatoms construct new frustule cell walls. After a cell divides, the epitheca and hypotheca separate, one remaining with each new daughter cell. The two cells then produce a new hypotheca. Diatoms do reproduce sexually, but not with the same frequency.

See also Autotrophic bacteria; Fossilization of bacteria; Photosynthesis; Photosynthetic microorganisms; Plankton and planktonic bacteria

DICTYOSTELIUM

Dictyostelium discoideum, also know as slime **mold**, are single-celled soil amoeba which naturally occur amongst decaying leaves on the forest floor. Their natural food sources are **bacteria** that are engulfed by **phagocytosis**. Amoeba are eukaryotic organisms, that is, they organize their genes onto **chromosomes**. *Dictyostelium* may be either haploid (the vast majority) or diploid (approximately 1 in 10,000 cells).

There is no true sexual phase of development, although two haploid cells occasionally coalesce into a diploid organism. Diploid cells may lose chromosomes one by one to transition back to a haploid state. When food sources are plentiful, *D. discoideum* reproduces by duplicating its genome and dividing into two identical diploid daughter cells. Under starvation conditions, Dictyostelium enter an extraordinary alternate life cycle in which large populations of cells spontaneously aggregate and begin to behave much like a multicellular organism. Aggregation is initiated when a small proportion of cells emit pulses of cyclic AMP drawing in cells in the immediate vicinity. In this phase of the life cycle, groups of 100,000 cells coalesce and develop a surface sheath to form well-defined slugs (pseudoplasmodia), which can migrate together as a unit. As the pseudoplasmodium phase nears its end, cells near the tip of the slug begin to produce large quantities of cellulose that aids the slug in standing erect. This new phase is called culmination. At this stage, cells from the underlying mound move upward toward the vertical tip where they are encapsulated into spores forming the fruiting body. Spores then are dispersed into the environment where they can remain dormant until favorable conditions arise to resume the primary life mode as independent organisms. Spores are resistant to heat, dehydration, and lack of food sources. When a source of amino acids is detected in the environment, spores open longitudinally, releasing a small but normal functioning amoeba.

Dictyostelium are valuable biological model organisms for studying the principals of morphological development and signaling pathways.

See also Microbial genetics

DIFFUSION • *see* CELL MEMBRANE TRANSPORT

DiGEORGE SYNDROME • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

DILUTION THEORY AND TECHNIQUES

Dilution allows the number of living **bacteria** to be determined in suspensions that contain even very large numbers of bacteria.

The number of bacteria obtained by dilution of a **culture** can involve growth of the living bacteria on a solid growth source, the so-called dilution plating technique. The objective of dilution plating is to have growth of the bacteria on the surface of the medium in a form known as a **colony**. Theoretically each colony arises from a single bacterium. So, a value called the colony-forming unit can be obtained. The acceptable range of colonies that needs to be present is between 30 and 300. If there is less than 30 colonies, the sample has been diluted too much and there is too a great variation in the number of colonies in each milliliter (ml) of the dilution examined. Confidence cannot be placed in the result. Conversely, if there are more than 300 colonies, the over-crowded colonies cannot be distinguished from one another.

To use an example, if a sample contained 100 living bacteria per ml, and if a single milliliter was added to the growth medium, then upon incubation to allow the bacteria to grow into colonies, there should be 100 colonies present. If, however, the sample contained 1,000 living bacteria per ml, then plating a single ml onto the growth medium would produce far too many colonies to count. What is needed in the second case is an intervening step. Here, a volume is withdrawn from the sample and added to a known volume of fluid. Typically either one ml or 10 ml is withdrawn. These would then be added to nine or 90 ml of fluid, respectively. The fluid used is usually something known as a **buffer**, which is fluid that does not provide nutrients to the bacteria but does provide the ions needed to maintain the bacteria in a healthy state. The original culture would thus have been diluted by 10 times. Now, if a milliliter of the diluted suspension was added to the growth medium, the number of colonies should be one-tenth of 1,000 (= 100). The number of colonies observed is then multiplied by the dilution factor to yield the number of living bacteria in the original culture. In this example, 100 colonies multiplied by the dilution factor of 10 yields 1,000 bacteria per ml of the original culture.

In practice, more than a single ten-fold dilution is required to obtain a countable number of bacterial colonies. Cultures routinely contain millions of living bacteria per milliliter. So, a culture may need to be diluted millions of times. This can be achieved in two ways. The first way is known as serial dilution. An initial 10-times dilution would be prepared as above. After making sure the bacteria are evenly dispersed throughout, for example, 10 ml of buffer, one milliliter of the dilution would be withdrawn and added to nine milliliters of buffer. This would produce a 10-times dilution of the first dilution, or a 100-times dilution of the original culture. A milliliter of the second dilution could be withdrawn and added to another nine milliliters of buffer (1,000 dilution of the original culture) and so on. Then, one milliliter of each dilution can be added to separate plates of growth medium and the number of colonies determined after incubation. Those plates that contain between 30 and 300 colonies could be used to determine the number of living bacteria in the original culture.

The other means of dilution involves diluting the sample by 100 times each time, instead of 10 times. Taking one milliliter of culture or dilution and adding it to 99 ml of buffer accomplish this. The advantage of this dilution scheme is that dilution is obtained using fewer materials. However, the dilution steps can be so great that the countable range of 30-300 is missed, necessitating a repeat of the entire procedure.

Another dilution method is termed the "most probable number" method. Here, 10-fold dilutions of the sample are made. Then, each of these dilutions is used to inoculate tubes of growth medium. Each dilution is used to inoculate either a set of three or five tubes. After incubation the number of tubes that show growth are determined. Then, a chart is consulted and the number of positive tubes in each set of each sample dilution is used to determine the most probable number (MPN) of bacteria per milliliter of the original culture.

See also Agar and agarose; Laboratory techniques in microbiology; Qualitative and quantitative techniques in microbiology

DINOFLAGELLATES

Dinoflagellates are **microorganisms** that are regarded as algae. Their wide array of exotic shapes and, sometimes, armored appearance is distinct from other algae. The closest microorganism in appearance are the **diatoms**.

Dinoflagellates are single-celled organisms. There are nearly 2000 known living species. Some are bacterial in size, while the largest, *Noctiluca*, can be up to two millimeters in size. This is large enough to be seen by the unaided eye.

Ninety per cent of all known dinoflagellates live in the ocean, although freshwater species also exist. In fact, dinoflagellates have even been isolated from snow. In these environments, the organisms can exist as free-living and independent forms, or can take up residence in another organism. A number of photosynthetic dinoflagellates inhabit sponges, corals, jellyfish, and flatworms. The association is symbiotic. The host provides a protective environment and the growth of the dinoflagellates impart nutritive carbohydrates to the host.

As their name implies, flagella are present. Indeed, the term dinoflagellate means whirling flagella. Typically, there are two flagella. One of these circles around the body of the cell, often lying in a groove called the cingulum. The other flagellum sticks outward from the surface of the cell. Both flagella are inserted into the dinoflagellate at the same point. The arrangement of the flagella can cause the organism to move in a spiral trajectory.

The complex appearance, relative to other algae and **bacteria**, is carried onward to other aspects of dinoflagellate behavior and growth. Some dinoflagellates feed on other microorganisms, while others produce energy using photosyn-

thesis. Still other dinoflagellates can do both. The life cycle of the organisms is also complex, involving forms that are immobile and capable of movement and forms that are capable of sexual or asexual reproduction (bacteria, for example, reproduce asexually, by the self-replication of their genetic material and other constituents). Dinoflagellates are primarily asexual in reproduction.

Some dinoflagellates contain plates of cellulose that lie between the two surface membranes that cover the organism. These plates function as protective armor.

Dinoflagellates are noteworthy for several reasons. They are one of the bedrocks of the food chain, particularly in the oceans and lakes of the world. Their numbers can be so great that they are evident as a mass of color on the surface of the water. Sometimes satellite cameras can even visualize these blooms. This abundant growth can consume so much oxygen that survival of other species in the area is threatened. As well, some dinoflagellates can produce toxins that can find their way into higher species, particularly those such as shellfish that feed by filtering water through them. Paralytic shellfish poisoning, which harms the neurological system of humans, is an example of a malady associated with the consumption of clams, mussels, and oysters that are contaminated with dinoflagellate toxins known as saxitoxin and brevitoxin. Saxitoxin is extremely potent, exerting its effect on the neurological system at concentrations 10,000 times lower than that required by cocaine. Another example of a dinoflagellate-related malady is a disease called ciguatera, which results from eating toxin-contaminated fish.

A third distinctive feature of dinoflagellates concerns their **nucleus**. The deoxyribonucleic acid shares some features with the **DNA** of **eukaryotes**, such as the presence of repeated stretches of DNA. But, other eukaryotic features, such as the supportive structures known as histones, have as yet not been detected. Also, the amount of DNA in dinoflagellates is far greater than in eukaryotes. The nucleus can occupy half the volume of the cell.

As with other microorganisms, dinoflagellates have been present on the Earth for a long time. Fossils of *Arpylorus antiquus* have been found in rock that dates back 400 million years. And, fossils that may be dinoflagellate cysts have been found in rock that is almost two billion years old. Current thought is that dinoflagellates arose when a bacterium was swallowed but not digested by another microorganism. The bacteria became symbiotic with the organism that swallowed them. This explanation is also how mitochondria are thought to have arisen.

Dinoflagellates cysts are analogous to the cysts formed by other microorganisms. They function to protect the genetic material during periods when conditions are too harsh for growth. When conditions become more favorable, resuscitation of the cyst and growth of the dinoflagellate resumes.

Dinoflagellates are sometimes referred to as Pyrrhophyta, which means fire plants. This is because of their ability to produce biological luminescence, akin to that of the firefly. Often, these luminescent dinoflagellates can be seen in the wake of ocean-going ships at night.

See also Bioluminescence; Red tide; Snow blooms

DIPHTHERIA

Diphtheria is a potentially fatal, contagious bacterial disease that usually involves the nose, throat, and air passages, but may also infect the skin. Its most striking feature is the formation of a grayish membrane covering the tonsils and upper part of the throat.

Like many other upper respiratory diseases, diphtheria is most likely to break out during the winter months. At one time it was a major childhood killer, but it is now rare in developed countries because of widespread **immunization**. Since 1988, all confirmed cases in the United States have involved visitors or immigrants. In countries that do not have routine immunization against this infection, the mortality rate varies from 1.5% to 25%.

Persons who have not been immunized may get diphtheria at any age. The disease is spread most often by droplets from the coughing or sneezing of an infected person or carrier. The incubation period is two to seven days, with an average of three days. It is vital to seek medical help at once when diphtheria is suspected, because treatment requires emergency measures for adults as well as children.

The symptoms of diphtheria are caused by toxins produced by the diphtheria bacillus, *Corynebacterium diphtheriae* (from the Greek for "rubber membrane"). In fact, toxin production is related to infections of the bacillus itself with a particular **bacteria** virus called a phage (from **bacteriophage**; a virus that infects bacteria). The intoxication destroys healthy tissue in the upper area of the throat around the tonsils, or in open wounds in the skin. Fluid from the dying cells then coagulates to form the telltale gray or grayish green membrane. Inside the membrane, the bacteria produce an exotoxin, which is a poisonous secretion that causes the life-threatening symptoms of diphtheria. The exotoxin is carried throughout the body in the bloodstream, destroying healthy tissue in other parts of the body.

The most serious complications caused by the exotoxin are inflammations of the heart muscle (myocarditis) and damage to the nervous system. The risk of serious complications is increased as the time between onset of symptoms and the administration of antitoxin increases, and as the size of the membrane formed increases. The myocarditis may cause disturbances in the heart rhythm and may culminate in heart failure. The symptoms of nervous system involvement can include seeing double (diplopia), painful or difficult swallowing, and slurred speech or loss of voice, which are all indications of the exotoxin's effect on nerve functions. The exotoxin may also cause severe swelling in the neck ("bull neck").

The signs and symptoms of diphtheria vary according to the location of the infection. Nasal diphtheria produces few symptoms other than a watery or bloody discharge. On examination, there may be a small visible membrane in the nasal passages. Nasal infection rarely causes complications by itself, but it is a **public health** problem because it spreads the disease more rapidly than other forms of diphtheria.

Pharyngeal diphtheria gets its name from the pharynx, which is the part of the upper throat that connects the mouth and nasal passages with the larynx. This is the most common

form of diphtheria, causing the characteristic throat membrane. The membrane often bleeds if it is scraped or cut. It is important not to try to remove the membrane because the trauma may increase the body's absorption of the exotoxin. Other signs and symptoms of pharyngeal diphtheria include mild sore throat, fever of 101–102°F (38.3–38.9°C), a rapid pulse, and general body weakness.

Laryngeal diphtheria, which involves the voice box or larynx, is the form most likely to produce serious complications. The fever is usually higher in this form of diphtheria (103–104°F or 39.4–40°C) and the patient is very weak. Patients may have a severe cough, have difficulty breathing, or lose their voice completely. The development of a "bull neck" indicates a high level of exotoxin in the bloodstream. Obstruction of the airway may result in respiratory compromise and death.

The skin form of diphtheria, which is sometimes called cutaneous diphtheria, accounts for about 33% of diphtheria cases. It is found chiefly among people with poor **hygiene**. Any break in the skin can become infected with diphtheria. The infected tissue develops an ulcerated area and a diphtheria membrane may form over the wound but is not always present. The wound or ulcer is slow to heal and may be numb or insensitive when touched.

The diagnosis of diphtheria can be confirmed by the results of a **culture** obtained from the infected area. Material from the swab is put on a **microscope** slide and stained using a procedure called **Gram's stain**. The diphtheria bacillus is called Gram-positive because it holds the dye after the slide is rinsed with alcohol. Under the microscope, diphtheria bacilli look like beaded rod-shaped cells, grouped in patterns that resemble Chinese characters. Another laboratory test involves growing the diphtheria bacillus on Loeffler's medium.

The most important treatment is prompt administration of diphtheria antitoxin. The antitoxin is made from horse serum and works by neutralizing any circulating exotoxin. The physician must first test the patient for sensitivity to animal serum. Patients who are sensitive (about 10%) must be desensitized with diluted antitoxin, since the antitoxin is the only specific substance that will counteract diphtheria exotoxin. No human antitoxin is available for the treatment of diphtheria.

Antibiotics are given to wipe out the bacteria, to prevent the spread of the disease, and to protect the patient from developing **pneumonia**. They are not a substitute for treatment with antitoxin. Both adults and children may be given **penicillin**, ampicillin, or erythromycin. Erythromycin appears to be more effective than penicillin in treating people who are carriers because of better penetration into the infected area. Cutaneous diphtheria is usually treated by cleansing the wound thoroughly with soap and water, and giving the patient antibiotics for 10 days.

Universal immunization is the most effective means of preventing diphtheria. The standard course of immunization for healthy children is three doses of DPT (diphtheria-tetanus-pertussis) preparation given between two months and six months of age, with booster doses given at 18 months and at entry into school. Adults should be immunized at 10-year intervals with Td (tetanus-diphtheria) toxoid. A toxoid is a bacterial toxin that is treated to make it harmless but still can induce **immunity** to the disease.

Diphtheria patients must be isolated for one to seven days or until two successive cultures show that they are no longer contagious. Because diphtheria is highly contagious and has a short incubation period, family members and other contacts of diphtheria patients must be watched for symptoms and tested to see if they are carriers. They are usually given antibiotics for seven days and a booster shot of diphtheria/tetanus toxoid.

Reporting is necessary to track potential **epidemics**, to help doctors identify the specific strain of diphtheria, and to see if resistance to penicillin or erythromycin has developed. In 1990, an outbreak of diphtheria began in Russia and spread within four years to all of the newly independent states of the former Soviet Union. By the time that the epidemic was contained, over 150,000 cases and 5000 deaths were reported. A vast public health immunization campaign largely confined the epidemic by 1999.

See also Bacteria and bacterial infection; Epidemics, bacterial; Public health, current issues

DIRECT MICROSCOPIC COUNT • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

DISEASE OUTBREAKS • *see* EPIDEMICS AND PANDEMICS

DISINFECTION AND DISINFECTANTS

Disinfection and the use of chemical disinfectants is one key strategy of **infection control**. Disinfection refers to the reduction in the number of living **microorganisms** to a level that is considered to be safe for the particular environment. Typically, this entails the destruction of those microbes that are capable of causing disease.

Disinfection is different from **sterilization**, which is the complete destruction of all microbial life on the surface or in the liquid. The steam-heat technique of autoclaving is an example of sterilization.

There are three levels of disinfection, with respect to power of the disinfection. High-level disinfection will kill all organisms, except for large concentrations of bacterial spores, using a chemical agent that has been approved as a so-called sterilant by the United States Food and Drug Administration. Intermediate level disinfection is that which kills mycobacteria, most **viruses**, and all types of **bacteria**. This type of disinfection uses a chemical agent that is approved as a tuberculocide by the United States Environmental Protection Agency (EPA). The last type of disinfection is called low-level disinfection. In this type, some viruses and bacteria are killed using a chemical compound designated by the EPA as a hospital disinfectant.

There are a variety of disinfectants that can be used to reduce the microbial load on a surface or in a solution. The

disinfectant that is selected and the use of the particular disinfectant depend on a number of factors. The nature of the surface is important. A smoother surface is easier to disinfect, as there are not as many crevasses for organisms to hide. Generally, a smoother surface requires less time to disinfect than a rough surface. The surface material is also important. For example, a wooden surface can soak up liquids that can act as nutrients for the microorganisms, while a plastic surface that is more **hydrophobic** (water-hating) will tend to repel liquids and so present a more hostile environment for microbes.

Another factor in the **selection** of a disinfectant is the number of living microorganisms present. Generally, more organisms require a longer treatment time and sometimes a more potent disinfectant. The nature of the microbial growth is also a factor. Bacteria growing a slime-encased **biofilm** are hardier than bacteria that are not growing in biofilms. Other resistance mechanisms can operate. A general order of resistance, from the most to the least resistant, is: bacterial spores, mycobacteria (because of their unusual cell wall composition), viruses that repel water, **fungi**, actively growing bacteria, and viruses whose outer surface is mostly lipid.

Alcohol is a disinfectant that tends to be used on the skin to achieve a short-term disinfection. It can be used on surfaces as a spray. However, because alcohol evaporates quickly, it may not be present on a surface long enough to adequately disinfect the surface. A type of disinfectant known as tamed iodines, or iodophors, are also useful as skin disinfectants. In hospital settings, iodophors are used as a replacement for hand soap.

A better choice of disinfectant for surfaces is sodium hypochlorite. It can also be added to drinking water, where dissociation to produce free chlorine provides disinfection power. Bacteria such as ***Escherichia coli*** are susceptible to chlorine. **Chlorination** of drinking water is the most popular choice of water treatment in the world. If left for five minutes, sodium hypochlorite performs as an intermediate level disinfectant on surfaces. However, chlorine bleach can be corrosive to metal surfaces and irritating to mucous membranes of the eye and nose.

Another surface disinfectant is compounds that contain a phenol group. A popular commercial brand known as Lysol is a phenolic disinfectant. Phenolics are intermediate level disinfectants, derived from coal tar, that are effective on contaminated surfaces. However, certain types of viruses and some bacteria are resistant to the killing action of phenolic compounds.

Another disinfectant is chlorhexidine. It is effective against fungus and **yeast**, but is not as effective against Gram-negative bacteria. Nor will it inactivate viruses whose surfaces are water loving. In situations where the contaminant is expected to be fungi or yeast, chlorhexidine is a suitable choice of disinfectant.

Aldehyde compounds, such as formaldehyde and glutaraldehyde, are very effective disinfectants. Glutaraldehyde has other uses as well, such as preserving specimens prior to their examination by the technique of electron microscopy. Glutaraldehyde kills many microorganisms, and all known disease-causing microorganisms, after only a few minutes exposure. Another effective general disinfectant is those that contain quaternary ammonium.

Disinfection of hands.

Many disinfectants are non-specific in their action. They will act against any biological material that is present. These are referred to as broad-spectrum disinfectants. Examples of broad-spectrum disinfectants are glutaraldehyde, sodium hypochlorite (the active ingredient in common household bleach), and hydrogen peroxide. Disinfectants such as phenolics and quaternary ammonium compounds are very specific. Other disinfectants lie in between the highly specific and broadly based categories. For example, alcohol is effective against actively growing bacteria and viruses with a lipid-based outer surface, but is not effective against bacterial spores or viruses that prefer watery environments.

The potency of a disinfectant can also be affected by the concentration that is used. For example, pure alcohol is less effective than alcohol diluted with water, because the more dilute form can penetrate farther into biological specimens than the pure form can.

Another factor that can decrease the effectiveness of disinfectants can be the presence of organic (carbon-containing) material. This can be a great problem in the chlorine disinfection of surface water. The vegetation in the water can bind the chlorine, leaving less of the disinfectant available to act on the microorganisms in the water. Proteins can also bind disinfectants. So, the presence of blood or blood products,

other body fluids, and fecal waste material can compromise disinfectant performance.

Microorganisms can develop resistance to disinfectants, or can even have built-in, or intrinsic, resistance. For example, application of some disinfectants to contaminated surfaces for too short a time can promote the development of resistance in those bacteria that survive the treatment.

See also Bacteriocidal and bacteriostatic; Fungicide

DISPOSAL OF INFECTIOUS MICROORGANISMS

In research and clinical settings, the safe disposal of **microorganisms** is of paramount importance. Microbes encountered in the hospital laboratory have often been isolated from patients. These organisms can be the cause of the malady that has hospitalized the patient. Once examination of the microorganisms has ended, they must be disposed of in a way that does not harm anyone in the hospital or in the world outside of the hospital. For example, if solutions of the living microorganisms were simply dumped down the sink, the infectious organisms could find their way to the water table, or could become aerosolized and infect those who happened to inhale the infectious droplets.

A similar scenario operates in the research laboratory. Research can involve the use of hazardous microorganisms. Facilities can be constructed to minimize the risk to researchers who work with the organisms, such as fume hoods, glove boxes and, in special circumstances, whole rooms designed to contain the microbes. However, steps need to be taken to ensure that the organisms that are disposed of no longer present a risk of infection.

In addition to the cultures of microorganisms, anything that the organisms contacted must be disposed of carefully. Such items include tissues, syringes, the bedding in animal cages, **microscope** slides, razors, and pipettes. Often glassware and syringe are disposed of in sturdy plastic containers, which can be sterilized. The so-called "sharps" container prevents the sharp glass or syringe tip from poking out and cutting those handling the waste.

Depending on the material, there are several means by which items can be treated. The most common methods of treatment and disposal are **disinfection** using chemicals, **sterilization** using steam (such as in an autoclave), and burning at high temperature (which is also called incineration).

Disinfection can be done using chemicals. For example, a common practice in a microbiology laboratory is to wipe off the lab bench with alcohol both before and after a work session. Other liquid chemicals that are used as disinfectants include formaldehyde and chlorine-containing compounds (that are commonly referred to as bleach). Chemical disinfection can be achieved using a gas. The most common example is the use of ethylene oxide. Gas disinfection is advantageous when the sample is such that scrubbing of inner surfaces cannot be done, such as in tubing.

Biohazard technician handles suspected infectious microorganisms.

A second means of waste treatment is sterilization. This is the complete elimination of living organisms. A very common means of sterilization is the use of steam. The most common form of steam sterilization in laboratory settings is the autoclave. For example, in disinfection procedures and other laboratory procedures, items such as the adsorbent material used to wipe the bench and plastic gloves are usually put into a special biohazard bag. The bag is sealed when it is full and is sterilized, typically in an autoclave. The seal is typically an indicator tape that displays marking if the sterilization conditions have been achieved. The inclusion in the load being autoclaved of a solution containing spores of *Bacillus sterothermophilus* is typically done at regular intervals. Attempts to grow the contents of the solution after autoclaving should be unsuccessful if the sterilization procedure worked. After successful sterilization, the bag can be treated as normal waste.

An autoclave is essentially a large pressure cooker. Samples to be treated are placed in a chamber and a door can be tightly sealed. The seal is so tight that air cannot escape. Steam is introduced into the chamber at high pressure. At higher pressure a higher temperature can be achieved than the 100° C [212° F] possible at atmospheric pressure.

The relationship between time and temperature determines the speed of sterilization. The higher the temperature the more quickly a sample can be sterilized. Typical combinations of temperature and pressure are 115° C [239° F]–10 pounds per square inch (psi), 121° C [249.8° F]–15 psi, and 132° C [269.6° F]–27psi. Which combination is used depends on the material being sterilized. For example, a large and bulky load, or a large volume of **culture** should be kept in longer. Shorter sterilizations times are sufficient for contaminated objects such as surgical dressing, instruments, and empty glassware.

The third method of treatment of microorganisms and material contaminated with microorganisms is incineration. On a small scale incineration is practiced routinely in a microbiology laboratory to sterilize the metal loops used to transfer microorganisms from one place to another. Exposing the metal loop to a gas flame will burn up and vaporize any living microbes that are on the loop, ensuring that infectious organisms are not inadvertently transferred elsewhere. The method of incineration is also well suited to the treatment of large volumes of contaminated fluids or solids. Incineration is carried out in specially designed furnaces that achieve high temperatures and are constructed to be airtight. The use of a flame source such as a fireplace is unsuitable. The incineration needs to occur very quickly and should not leave any residual material. The process needs to be smoke-free, otherwise microbes that are still living could be wafted away in the rising smoke and hot air to cause infection elsewhere. Another factor in proper incineration is the rate at which sample is added to the flame. Too much sample can result in an incomplete burn.

Disposal of microorganisms also requires scrupulous record keeping. The ability to back track and trace the disposal of a sample is very important. Often institutions will have rules in place that dictate how samples should be treated, the packaging used for disposal, the labeling of the waste, and the records that must be maintained.

See also Laboratory techniques in microbiology; Steam pressure sterilizer

DNA (DEOXYRIBONUCLEIC ACID)

DNA, or deoxyribonucleic acid, is the genetic material that codes for the components that make life possible. Both prokaryotic and eukaryotic organisms contain DNA. An exception is a few **viruses** that contain **ribonucleic acid**, although even these viruses have the means for producing DNA.

The DNA of **bacteria** is much different from the DNA of eukaryotic cells such as human cells. Bacterial DNA is dispersed throughout the cell, while in eukaryotic cells the DNA is segregated in the **nucleus**, a membrane-bound region. In eukaryotics, structures called mitochondria also contain DNA. The dispersed bacterial DNA is much shorter than eukaryotic DNA. Hence the information is packaged more tightly in bacterial DNA. Indeed, in DNA of **microorganisms** such as viruses, several genes can overlap with each other, providing information for several proteins in the same stretch of nucleic acid. Eukaryotic DNA contains large intervening regions between genes.

The DNA of both prokaryotes and **eukaryotes** is the basis for the transfer of genetic traits from one generation to the next. Also, alterations in the genetic material (**mutations**) can produce changes in structure, **biochemistry**, or behavior that might also be passed on to subsequent generations.

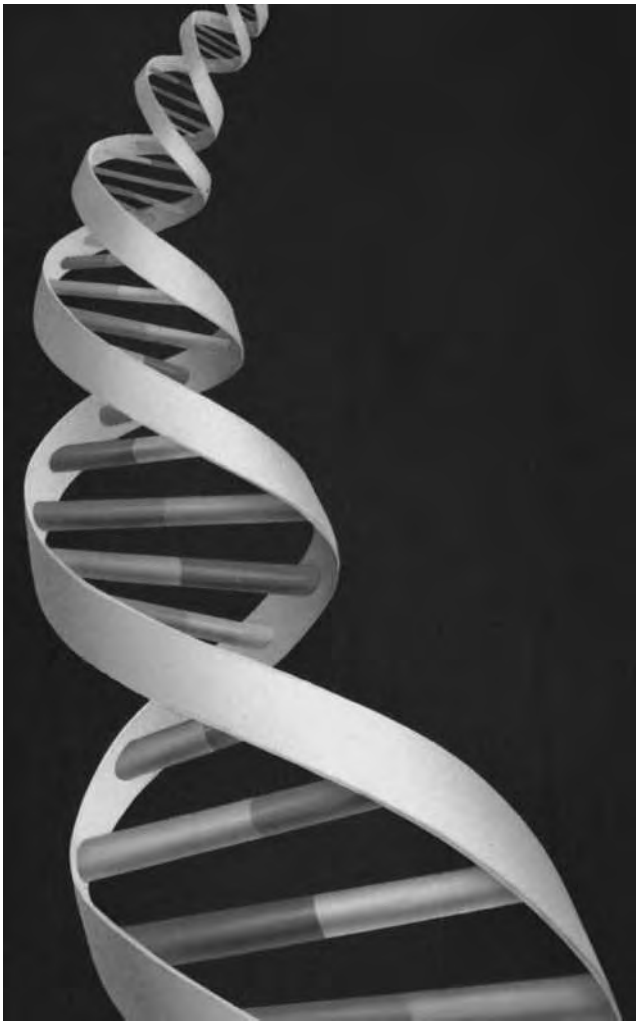
Genetics is the science of heredity that involves the study of the structure and function of genes and the methods by which genetic information contained in genes is passed from one generation to the next. The modern science of genetics can be traced to the research of Gregor Mendel (1823–1884), who was able to develop a series of laws that described mathematically the way hereditary characteristics pass from parents to offspring. These laws assume that hereditary characteristics are contained in discrete units of genetic material now known as genes.

The story of genetics during the twentieth century is, in one sense, an effort to discover the **gene** itself. An important breakthrough came in the early 1900s with the work of the American geneticist, Thomas Hunt Morgan (1866–1945). Working with fruit flies, Morgan was able to show that genes are somehow associated with the **chromosomes** that occur in the nuclei of cells. By 1912, Hunt's colleague, American geneticist A. H. Sturtevant (1891–1970) was able to construct the first chromosome map showing the relative positions of different genes on a chromosome. The gene then had a concrete, physical referent; it was a portion of a chromosome.

During the 1920s and 1930s, a small group of scientists looked for a more specific description of the gene by focusing their research on the gene's molecular composition. Most researchers of the day assumed that genes were some kind of protein molecule. Protein molecules are large and complex. They can occur in an almost infinite variety of structures. This quality is expected for a class of molecules that must be able to carry the enormous variety of genetic traits.

A smaller group of researchers looked to a second family of compounds as potential candidates as the molecules of heredity. These were the nucleic acids. The nucleic acids were first discovered in 1869 by the Swiss physician Johann Miescher (1844–1895). Miescher originally called these compounds "nuclein" because they were first obtained from the nuclei of cells. One of Miescher's students, Richard Altmann, later suggested a new name for the compounds, a name that better reflected their chemical nature: nucleic acids.

Nucleic acids seemed unlikely candidates as molecules of heredity in the 1930s. What was then known about their structure suggested that they were too simple to carry the vast array of complex information needed in a molecule of heredity. Each nucleic acid molecule consists of a long chain of alternating sugar and phosphate fragments to which are attached some sequence of four of five different nitrogen bases: adenine, cytosine, guanine, uracil and thymine (the exact bases found in a molecule depend slightly on the type of nucleic acid).



Computer-generated image of the DNA double helix, showing the deoxyribose backbone (vertical ribbons) and the linking nucloetides (horizontal bars).

It was not clear how this relatively simple structure could assume enough different conformations to "code" for hundreds of thousands of genetic traits. In comparison, a single protein molecule contains various arrangements of twenty fundamental units (amino acids) making it a much better candidate as a carrier of genetic information.

Yet, experimental evidence began to point to a possible role for nucleic acids in the transmission of hereditary characteristics. That evidence implicated a specific sub-family of the nucleic acids known as the deoxyribose nucleic acids, or DNA. DNA is characterized by the presence of the sugar deoxyribose in the sugar-phosphate backbone of the molecule and by the presence of adenine, cytosine, guanine, and thymine, but not uracil.

As far back as the 1890s, the German geneticist Albrecht Kossel (1853–1927) obtained results that pointed to the role of DNA in heredity. In fact, historian John Gribbin has suggested that the evidence was so clear that it "ought to have been enough alone to show that the hereditary information...*must* be carried by the DNA." Yet, somehow, Kossel himself did not see this point, nor did most of his colleagues for half a century.

As more and more experiments showed the connection between DNA and genetics, a small group of researchers in the 1940s and 1950s began to ask how a DNA molecule could code for genetic information. The two who finally resolved this question were **James Watson**, a 24-year-old American trained in genetics, and **Francis Crick**, a 36-year-old Englishman, trained in physics and self-taught in chemistry. The two met at the Cavendish Laboratories of Cambridge University in 1951. They shared the view that the structure of DNA held the key to understanding how genetic information is stored in a cell and how it is transmitted from one cell to its daughter cells.

The key to lay in a technique known as x-ray crystallography. When x rays are directed at a crystal of some material, such as DNA, they are reflected and refracted by atoms that make up the crystal. The refraction pattern thus produced consists of a collection of spots and arcs. A skilled observer can determine from the refraction pattern the arrangement of atoms in the crystal.

Watson and Crick were fortunate in having access to some of the best x-ray diffraction patterns that then existed. These "photographs" were the result of work being done by **Maurice Wilkins** and Rosalind Elsie Franklin at King's College in London. Although Wilkins and Franklin were also working on the structure of DNA, they did not recognize the information their photographs contained. Indeed, it was only when Watson accidentally saw one of Franklin's photographs that he suddenly saw the solution to the DNA puzzle.

Watson and Crick experimented with tinker-toy-like models of the DNA molecule, shifting atoms around into various positions. They were looking for an arrangement that would give the kind of x-ray photograph that Watson had seen in Franklin's laboratory. On March 7, 1953, the two scientists found the answer. They built a model consisting of two helices (corkscrew-like spirals), wrapped around each other. Each helix consisted of a backbone of alternating sugar and phosphate groups. To each sugar was attached one of the four nitrogen bases, adenine, cytosine, guanine, or thymine. The sugar-phosphate backbone formed the outside of the DNA molecule, with the nitrogen bases tucked inside. Each nitrogen base on one strand of the molecule faced another nitrogen base on the opposite strand of the molecule. The base pairs were not arranged at random, however, but in such a way that each adenine was paired with a thymine, and each cytosine with a guanine.

The Watson-Crick model was a remarkable achievement, for which the two scientists won the 1954 Nobel Prize in Chemistry. The molecule had exactly the shape and dimensions needed to produce an x-ray photograph like that of Franklin's. Furthermore, Watson and Crick immediately saw how the molecule could "carry" genetic information. The sequence of nitrogen bases along the molecule, they said, could act as a **genetic code**. A sequence, such as A-T-T-C-G-C-T...etc., might tell a cell to make one kind of protein (such

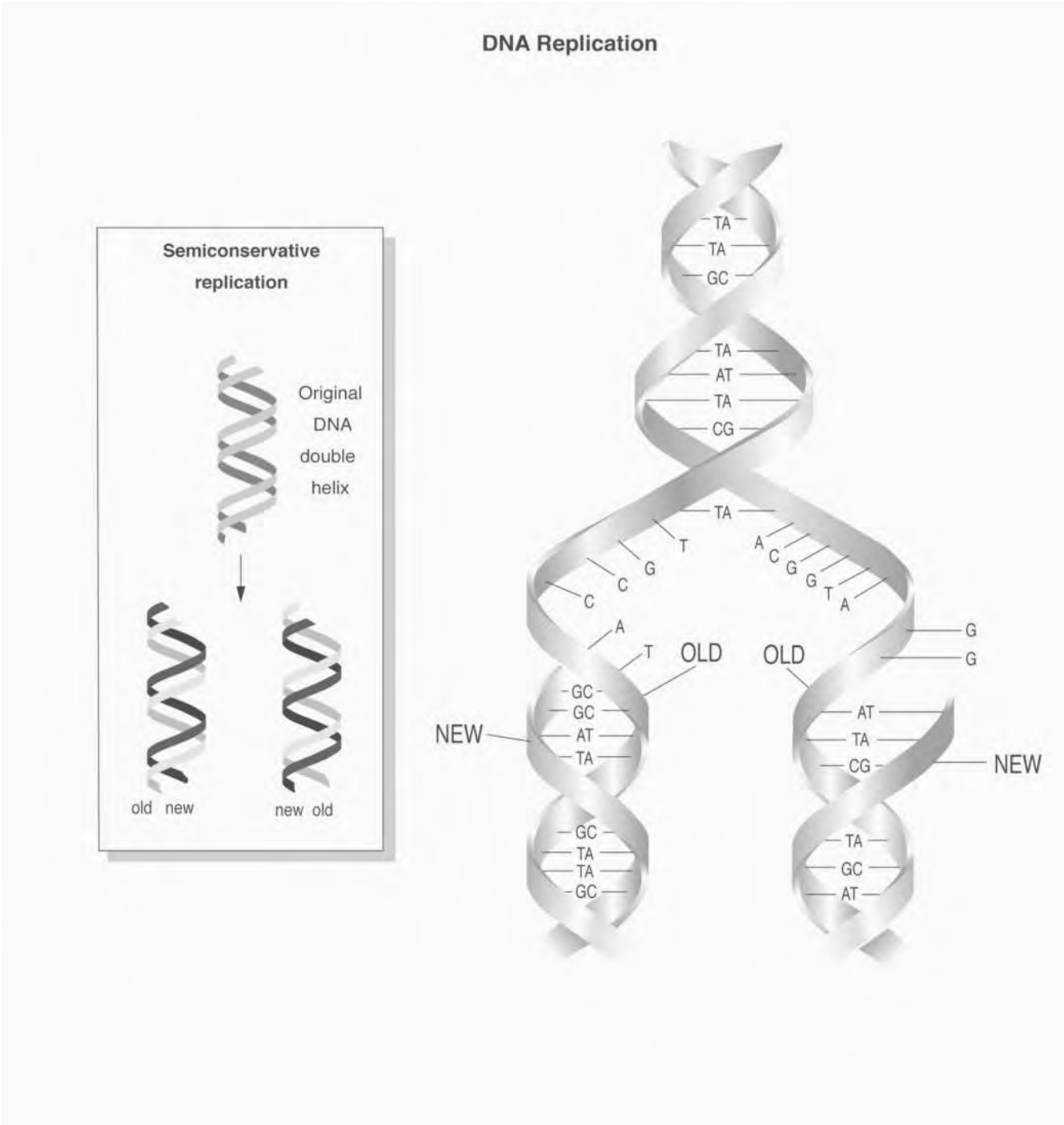


Diagram depicting semiconservative DNA replication.

as that for red hair), while another sequence, such as G-C-T-C-T-C-G...etc., might code for a different kind of protein (such as that for blonde hair). Watson and Crick themselves contributed to the deciphering of this genetic code, although that process was long and difficult and involved the efforts of dozens of researchers over the next decade.

Watson and Crick had also considered, even before their March 7th discovery, what the role of DNA might be in the manufacture of proteins in a cell. The sequence that they outlined was that DNA in the nucleus of a cell might act as a template for the formation of a second type of nucleic acid, **RNA** (ribonucleic acid). RNA would then leave the nucleus, emigrate to the **cytoplasm** and then itself act as a template for the production of protein. That theory, now known as the Central Dogma, has since been

largely confirmed and has become a critical guiding principal of much research in **molecular biology**.

Scientists continue to advance their understanding of DNA. Even before the Watson-Crick discovery, they knew that DNA molecules could exist in two configurations, known as the "A" form and the "B" form. After the Watson-Crick discovery, two other forms, known as the "C" and "D" configurations were also discovered. All four of these forms of DNA are right-handed double helices that differ from each other in relatively modest ways.

In 1979, however, a fifth form of DNA known as the "Z" form was discovered by Alexander Rich and his colleagues at the Massachusetts Institute of Technology. The "Z" form was given its name partly because of its zigzag shape and partly because it is different from the more common A and B forms. Although Z-DNA was first recognized in synthetic DNA prepared in the laboratory, it has since been found in natural cells whose environment is unusual in some respect or another. The presence of certain types of proteins in the nucleus, for example, can cause DNA to shift from the B to the Z conformation. The significance and role of this most recently discovered form of DNA remains a subject of research among molecular biologists.

See also Chemical mutagenesis; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Mitochondrial DNA

DNA CHIPS AND MICROARRAYS

A **DNA** (**deoxyribonucleic acid**) chip is a solid support (typically glass or nylon) onto which are fixed single strands of DNA sequences. The sequences are made synthetically and are arranged in a pattern that is referred to as an array. DNA chips are a means by which a large amount of DNA can be screened for the presence of target regions. Furthermore, samples can be compared to compare the effects of a treatment, environmental condition, or other factor on the activity. One example of the use of a DNA microarray is the screening for the development of a mutation in a **gene**. The original gene would be capable of binding to the synthetic DNA target, whereas the mutated gene does not bind. Such an experiment has been exploited in the search for genetic determinants of **antibiotic resistance**, and in the manufacture of compounds to which the resistant **microorganisms** will be susceptible.

A gene chip is wafer-like in appearance, and resembles a microtransistor chips. However, instead of transistors, a DNA chip contains an orderly and densely packed array of DNA species. Arrays are made by spotting DNA samples over the surface of the chip in a patterned manner. The spots can be applied by hand or with robotic automation. The latter can produce very small spots, which collectively is termed a microarray.

Each spot in an array is, in reality, a single-stranded piece of DNA. Depending upon the sequence of the tethered piece of DNA, a complimentary region of sample DNA can specifically bind. The design of the array is dependent on the nature of the experiment.

The synthetic DNA is constructed so that known sequences are presented to whatever sample is subsequently applied to the chip. DNA, or **ribonucleic acid** (typically messenger **RNA**) from the samples being examined are treated to as to cut the double helix of DNA into its two single strand components, following be enzymatic treatment that cuts the DNA into smaller pieces. The pieces are labeled with **fluorescent dyes**. For example, the DNA from one sample of **bacteria** could be tagged with a green fluorescent dye (dye that will fluoresce green under illumination with a certain wavelength of light) and the DNA from a second sample of bacteria could be tagged with a red fluorescent dye (which will fluoresce red under illumination with the same wavelength of light). Both sets of DNA are flooded over the chip. Where the sample DNA finds a complimentary piece of synthetic DNA, binding will occur. Finally the nature of the bound sample DNA is ascertained by illuminating the chip and observing for the presence and the pattern of green and red regions (usually dots).

A microarray can also be used to determine the level of expression of a gene. For example, an array can be constructed such that the messenger RNA of a particular gene will bind to the target. Thus, the bound RNAs represent genes that were being actively transcribed, or at least recently. By monitoring genetic expression, the response of microorganisms to a treatment or condition can be examined. As an example, DNA from a bacterial species growing in suspension can be compared with the same species growing as surface-adherent biofilm in order to probe the genetic nature of the alterations that occur in the bacteria upon association with a surface. Since the method detects DNA, the survey can be all-encompassing, assaying for genetic changes to protein, carbohydrate, lipid, and other constituents in the same experiment.

The power of DNA chip technology has been recently illustrated in the Human Genome Project. This effort began in 1990, with the goal of sequencing the complete human genome. The projected time for the project's completion was 40 years. Yet, by 2001, the sequencing was essentially complete. The reason for the project's rapid completion is the development of the gene chip.

Vast amounts of information are obtained from a single experiment. Up to 260,000 genes can be probed on a single chip. The analysis of this information has spawned a new science called **bioinformatics**, where biology and computing mesh.

Gene chips are having a profound impact on research. Pharmaceutical companies are able to screen for gene-based drugs much faster than before. In the future, DNA chip technology will extend to the office of the family physician. For example, a patient with a sore throat could be tested with a single-use, disposable, inexpensive gene chip in order to identify the source of the infection and its antibiotic susceptibility profile. Therapy could commence sooner and would be precisely targeted to the causative infectious agent.

See also DNA (Deoxyribonucleic acid); DNA chips and micro arrays; DNA hybridization; Genetic identification of microor-

ganisms; Laboratory techniques in immunology; Laboratory techniques in microbiology; Molecular biology and molecular genetics

DNA Hybridization

Evolution deals with heritable changes in populations over time. Because **DNA** is the molecule of heredity, evolutionary changes will be reflected in changes in the base pairs in DNA. Two species that have evolved from a common ancestor will have DNA that has very similar base pair sequences. The degree of relatedness of two species can be estimated by examining how similar their base pair sequences are. One method of assessing relatedness uses hybridization of DNA.

In the molecular genetic technique of hybridization of DNA, single strands of DNA from two different species are allowed to join together to form hybrid double helices. These hybrid segments of DNA can be used to determine the evolutionary relatedness of organisms by examining how similar or dissimilar the DNA base pair sequences are.

The technique of DNA hybridization is based on two principles: the first, that double strands of DNA are held together by hydrogen bonds between complementary base pairs, and the second is that the more closely related two species are, the greater will be the number of complementary base pairs in the hybrid DNA. In other words, the degree of hybridization is proportional to the degree of similarity between the molecules of DNA from the two species.

Hybridization of DNA is accomplished by heating strands of DNA from two different species to 86° C [186.8° F]. This breaks the hydrogen bonds between all complementary base pairs. The result is many single-stranded segments of DNA. The single-stranded DNA from both species is mixed together and allowed to slowly cool. Similar strands of DNA from both species will begin to chemically join together or re-anneal at complementary base pairs by reforming hydrogen bonds.

The resulting hybrid DNA is then reheated and the temperature at which the DNA once again becomes single-stranded is noted. Because one cannot observe DNA separating, another technique must be used simultaneously with heating to show when separation has occurred. This technique employs the absorption of UV light by DNA. Single strands of DNA absorb UV light more effectively than do double strands. Therefore, the separation of the DNA strands is measured by UV light absorption; as more single strands are liberated, more UV light is absorbed.

The temperature at which hybrid DNA separation occurs is related to the number of hydrogen bonds formed between complementary base pairs. Therefore, if the two species are closely related, most base pairs will be complementary and the temperature of separation will be very close to 86° C [186.8° F]. If the two species are not closely related, they will not share many common DNA sequences and fewer complementary base pairs will form. The temperature of separation will be less than 86° C [186.8° F] because less energy is required to break fewer hydrogen bonds. Using this type of information, a tree of evolutionary relationships based on the separation temperature of the hybrid helices can be generated.

See also Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses

Donnan, Frederick George (1870-1956)

British chemist

Frederick George Donnan was a British chemist whose work in the second decade of the twentieth century established the existence of an electrochemical potential between a semipermeable membrane. The membrane allows an unequal distribution of ionic species to become established on either side of the membrane. In **bacteria**, this **Donnan equilibrium** has been demonstrated to exist across the outer membrane of Gram-negative bacteria, which separates the external environment from the **periplasm**. The energy derived from this ionic inequity is vital for the operation of the bacteria.

Donnan was born in Colombo, Ceylon (now known as Sri Lanka). He was educated at Queen's College in Belfast, Northern Ireland, at the University of Leipzig in Berlin, and at the University College, London. He taught at Liverpool University from 1904 until 1913, when he rejoined the faculty of University College as a Professor of Inorganic and Physical Chemistry. He remained there until his retirement in 1937.

In 1911, Donnan began his studies of the equilibrium between solutions separated by a semipermeable membrane that led to the establishment of the Donnan equilibrium. He also was involved in important studies in physical chemistry, which included the study of colloids and soap solutions, behavior of various gases, oxygen solubility, and the manufacture of nitric acid.

Of all his research achievements, Donnan's major accomplish was the theory of membrane equilibrium. In his productive research career, Donnan authored more than one hundred research papers.

See also Bacterial membranes and cell wall

Donnan equilibrium

Donnan equilibrium (which can also be referred to as the Gibbs-Donnan equilibrium) describes the equilibrium that exists between two solutions that are separated by a membrane. The membrane is constructed such that it allows the passage of certain charged components (ions) of the solutions. The membrane, however, does not allow the passage of all the ions present in the solutions and is thus a selectively permeable membrane.

Donnan equilibrium is named after **Frederick George Donnan**, who proved its existence in biological cells. J. Willard Gibbs had predicted the effect some 30 years before.

The impermeability of the membrane is typically related to the size of the particular ion. An ion can be too large to pass through the pores of the membrane to the other side. The concentration of those ions that can pass freely though the membrane is the same on both sides of the membrane. As well, the total number of charged molecules on either side of the membrane is equal.

A consequence of the selective permeability of the membrane barrier is the development of an electrical potential between the two sides of the membrane. The two solutions vary in osmotic pressure, with one solution having more of a certain type (species) or types of ion that does the other solution.

As a result, the passage of some ions across the membrane will be promoted. In **bacteria**, for example, the passage of potassium across the outer membrane of Gram-negative bacteria occurs as a result of an established Donnan equilibrium between the external environment and the **periplasm** of the bacterium. The potassium enters in an attempt to balance the large amount of negative ion inside the cell. Since potassium is freely permeable, it will tend to diffuse out again. The inward movement of sodium corrects the imbalance. In the absence of a Donnan equilibrium, the bulky sodium molecule would not normally tend to move across the membrane and an electrical potential would be created.

See also Biochemistry

DOOLITTLE, W. FORD (1942-)

American biochemist and evolutionary biologist

Ford Doolittle is a Professor in the Department of **Biochemistry** at Dalhousie University in Halifax, Nova Scotia, Canada. He is also Director of the Canadian Institute of Advanced Research Program in Evolutionary Biology. Doolittle is one of the world's premier evolutionary biologists, who has used molecular techniques to explore the similarities and disparities between the genetic material in a variety of prokaryotic and eukaryotic organisms. In particular, his pioneering studies with the evolutionarily ancient archaebacteria have led to a fundamental re-evaluation of the so-called "tree of life."

Doolittle was born in Urbana, Illinois. Following his high school education, he received a B.A. in Biological Sciences (magna cum laude) from Harvard College in 1963, and a Ph.D. in Biological Sciences from Stanford University in 1969. He was a Postdoctoral Fellow in Microbiology at the University of Illinois from 1968 to 1969, and at the National Jewish Hospital and Research Center in Denver from 1969 to 1971. From there he moved to Dalhousie University as an Assistant Professor in the Department of Biochemistry in 1971. He became an Associate Professor in 1976, and a Professor in 1982.

Doolittle and his colleagues have made fundamental contributions to the field of evolutionary biology. Specifically, Doolittle has pioneered studies examining the origin of the nuclear genetic material in eukaryotic cells, the origin of the organizing genetic material known as introns, and the genetic organization and regulation of the archaebacteria that inhabit thermal hot springs. The latter **bacteria** are among the most ancient **microorganisms** known, and knowledge of their genetic composition and behavior has clarified the early events of **evolution**.

From Doolittle's research, it is now known that mitochondria, the so-called "powerhouse" of eukaryotic cells, were once autonomous bacteria. Mitochondria arose from the integration of the ancient bacteria and a eukaryote and the establishment of a symbiotic relationship between the two. In addition, prokaryotic cells may well have evolved by acquiring genes from other species, even **eukaryotes**. This concept, which Doolittle has dubbed lateral **gene** transfer, challenges a fundamental pillar of evolution, which is the separateness of the kingdoms of life. For example, a fundamental scientific opposition to genetically modified organisms is that the acquisition of eukaryotic genes by the altered bacteria violates evolutionary laws.

Doolittle has received numerous awards and honors for his research, including the Award of Excellence from the Genetics Society of Canada and a fellowship in the Royal Society of Canada.

See also Archaeobacteria; Bacterial kingdoms; Evolutionary origin of bacteria and viruses

DUBOS, RENÉ (1901-1982)

French-born American microbiologist

René Dubos was a distinguished microbiologist whose pioneering work with soil-dwelling **bacteria** paved the way for the development of life-saving antibiotic drugs. Widely acclaimed for his discovery of tyrothricin, a chemical substance capable of destroying dangerous staphylococcus, pneumococcus, and streptococcus bacteria in both humans and animals, Dubos later turned to the study of **tuberculosis** and the role of physiological, social, and environmental factors in an individual's susceptibility to infection. In the 1960s, Dubos's interest in the effects of the total environment on human health and well-being prompted him to give up his laboratory work at New York's Rockefeller Institute for Medical Research to concentrate on writing and lecturing on ecological and humanitarian issues.

Over the years, Dubos produced a number of popular books on scientific subjects, including *So Human an Animal,* the 1968 Pulitzer-Prize winner for general nonfiction, and *Only One Earth: The Care and Maintenance of a Small Planet,* which formed the basis for the United Nations Conference on the Human Environment in 1992. Dubos's greatest concern was not man's inability to adapt to pollution, noise, overcrowding, and the other problems of highly industrialized societies, but rather the ease with which this adaptation could occur and its ensuing cost to humanity. "It is not man the ecological crisis threatens to destroy, but the quality of human life," Dubos wrote in *Life* magazine. "What we call humanness is the expression of the interplay between man's

nature and the environment, an interplay which is as old as life itself and which is the mechanism for creation on Earth."

Dubos was born in Saint-Brice-sous-Foret, France, the only child of Georges Alexandre and Adeline Madeleine de Bloedt Dubos. Young Dubos spent his early years in the farming villages of Ile-de-France, north of Paris. Amongst the rolling hills and agricultural fields, Dubos developed a keen appreciation for the influence of landscape on the human spirit, a subject that would come to dominate his thoughts in later years. A bout with rheumatic fever at the age of ten both restricted Dubos's physical activity and enhanced his contemplative nature. When Dubos was 13, his father moved the family to Paris to open a butcher shop; a few months later, Georges Dubos was called to military service in World War I, leaving his wife and young son in charge of the business. Despite the best efforts of mother and son, the shop did poorly and the family had a difficult time getting by. Upon completing high school at the College Chaptal in 1919, Dubos had hoped to study history at the university, but the death of his father from head injuries suffered at the front forced him to stay closer to home to look after his mother. Dubos was granted a scholarship to study agricultural science at the Institut National Agronomique in Paris, receiving his bachelor of science degree in 1921. He spent part of the next year as an officer trainee in the French Army, but was soon discharged because of heart problems.

In 1922, Dubos was offered the job of assistant editor at a scholarly journal called *International Agriculture Intelligence,* published by the International Institute of Agriculture, then part of the League of Nations in Rome. Not long after he arrived in Italy, Dubos came across an article on soil microbes written by the Russian bacteriologist Sergei Winogradsky, who was then associated with the Pasteur Institute in Paris. Winogradsky's contention that microbes should be studied in their own environment rather than in pure, laboratory-grown cultures so intrigued Dubos that he resolved to become a bacteriologist. "This is really where my scholarly life began," he told John Culhane in an interview for the *New York Times Magazine*. "I have been restating that idea in all forms ever since." Soon after, Dubos happened to meet the American delegate to the International Institute of Agriculture, who convinced him to pursue graduate studies in the United States.

In order to finance his trip, Dubos translated books on forestry and agriculture and gave guided tours of Rome to foreign visitors. He eventually set sail for New York in 1924. During the crossing, Dubos ran into **Selman Waksman**, head of the soil microbiology division of the State Agricultural Experiment Station at Rutgers University in New Jersey (a man the aspiring scientist had guided around Rome some months before). After the ship docked in New York, Waksman introduced Dubos to his colleagues at Rutgers University, helping the young man secure a research assistantship in soil microbiology. While serving as an instructor in bacteriology over the next three years, Dubos completed work on his doctorate. His thesis, published in 1927, focused on the ways in which various soil **microorganisms** work to decompose cellulose in paper.

Upon completing his work at Rutgers, Dubos left for the University of North Dakota at Fargo to accept a teaching position in the department of microbiology. Soon after he arrived, however, Dubos received a telegram from the Rockefeller Institute for Medical Research in New York City offering him a fellowship in the department of pathology and bacteriology. Dubos immediately packed his bags, in part because the offer involved work on a project begun by Rockefeller bacteriologist **Oswald T. Avery**. Avery and his colleagues had been searching for a substance that could break down the semi-cellulose envelope which protects pneumococci bacteria, the microorganisms responsible for lobar **pneumonia** in human beings, from attack by the body's defense mechanisms. Dubos's bold assertion that he could identify an enzyme capable of decomposing this complex polysaccharide capsule with minimal damage to the host had evidently impressed Avery. With the exception of a two-year period in the early 1940s when he served on the faculty at Harvard Medical School, Dubos remained at the Rockefeller Institute, renamed Rockefeller University in 1965, for the next 44 years.

Guided by the studies of renowned bacteriologist **Louis Pasteur**, who maintained that any organic substance that accumulated could be broken down by natural energy, Dubos spent his first two years at the Institute searching fields, bogs, and swamps for a bacterium or fungus that could attack and decompose the tough polysaccharide coat surrounding pneumococci bacteria. Unlike other scientific investigators, who used enriched laboratory solutions to cultivate bacteria and force them to produce **enzymes**, Dubos concocted a solution rich in capsular polysaccharide, which he spread over a variety of soils. In 1929, he succeeded in isolating a swamp-dwelling bacillus which, because of its need for nourishment in an energy-starved environment, had been compelled to produce an enzyme capable of decomposing the polysaccharide capsule and digesting the pneumococci within. The following year Dubos was able to demonstrate the value of this particular enzyme in fighting pneumococcal infections in both animals and humans. The discovery confirmed Dubos's belief that soil bacteria were an important source of anti-infectious agents, inspiring him to search for other disease-fighting microbes.

In 1939, Dubos announced the discovery of a substance called tyrothricin, which had proved effective in fighting staphylococcus, pneumococcus, and streptococcus infections. Produced by the soil microorganism *Bacillus brevis*, tyrothricin was later found to contain two powerful chemicals, gramicidin and tyrocidine, which, though too toxic for ingestion, found widespread application in the treatment of external conditions, such as infectious lesions in humans and udder infections in cows. Dubos's groundbreaking work prompted scientists from around the world to conduct a wide-ranging search for antibiotic substances in natural environments. This ultimately resulted in a reexamination of the therapeutic properties of penicillin—first discovered in a bread **mold** ten years earlier by Alexander Fleming—and led to the isolation of a variety of new **antibiotics**, including streptomycin and the tetracyclines.

The death of Dubos's first wife, Marie Louise Bonnet, from tuberculosis in 1942 had a profound effect upon the sci-

entist's career. "There seemed no reason," he recalled for Culhane. "Why should she get [tuberculosis] in this environment?" After spending two years as a professor of tropical medicine at Harvard Medical School, Dubos returned to the Rockefeller Institute to begin a full-scale investigation of tuberculosis and its causes. Until that time, scientists attempting to study tuberculosis bacilli had been hindered by the fact that laboratory methods of cultivation often modified the organisms to such an extent that they no longer resembled or behaved like the strains that infected humans. By 1947, however, Dubos had discovered that by adding a common detergent to the **culture** medium, he could raise bacilli so quickly and in such large quantities that they had little chance to mutate. This enabled researchers to study the microorganism more closely and develop the highly effective Bacillus Calmette-GuÉrin, or BCG, **vaccine**.

During the course of his research with tuberculosis, Dubos focused on the importance of heredity, nutrition, physiology, and social and emotional trauma on an individual's vulnerability to infection. He used his wife's case as his first example. A careful examination of her early health records revealed that she had suffered from tuberculosis as a child. Although his wife recovered from the acute attack, Dubos became convinced that the emotional upheaval of World War II and her concern for her family's safety in France had served to weaken her and reawaken the dormant germ. Some years later, Dubos's second wife's battle with tuberculosis and her subsequent recovery prompted the couple to collaborate on *The White Plague: Tuberculosis, Man, and Society,* a nontechnical account of the disease. Published in 1952, the book provided additional evidence linking tuberculosis with certain environmental conditions, such as inadequate nourishment and sudden economic or social disturbances.

Later, Dubos's interest in the effects of the total environment on human health encouraged him to become involved with the sociomedical problems of poor communities and to speak out on the dangers of pollution, as well as social, economic, and spiritual deprivation. By 1964, he had become a leading spokesman for the fledgling environmental movement and an outspoken critic of what he viewed as the narrow, short-range approach used by most biologists.

According to Dubos, the problems of technologically advanced societies posed an equal, if not greater, threat to human survival. Two of Dubos's most popular books, *Man Adapting* and *So Human an Animal,* examine the close relationship between environmental conditions and man's physical, mental, and spiritual development, emphasizing the dangers inherent in adapting to a polluted, highly mechanized, highly stressful environment. "Wild animals can survive in zoos, but only at the cost of losing the physical and behavioral splendor they possess in their natural habitat," he wrote in *Life*. "Similarly, human beings can survive in the polluted cage of technological civilization, but in adapting to such conditions, we may sacrifice much of our humanness." Dubos also warned against introducing new substances, such as laundry detergents containing potentially dangerous enzymes, into the American marketplace without thorough testing. Unlike many environmentalists, however, Dubos maintained an enormous faith in both the ability of nature to recover from man's abuses and man's own capacity to recognize and learn from mistakes.

Dubos became a naturalized American citizen in 1938. Although he maintained a laboratory and an apartment in New York City, Dubos spent most weekends at his large estate in Garrison, New York. There, he and his wife planted trees, raised vegetables, and enjoyed long walks in the scenic Hudson River Valley. Over the years, Dubos earned numerous awards for his work, including the Modern Medicine Award, 1961, the Phi Beta Kappa Award, 1963, and the Tyler Ecology Award, 1976; he also received more than thirty honorary degrees from various colleges and universities. A member of professional organizations such the National Academy of Sciences, Dubos was also appointed by President Richard M. Nixon in 1970 to serve on the Citizens' Advisory Committee on Environmental Quality. Always eager to make his scientific and philosophical ideas accessible to people from all walks of life, Dubos continued to write and lecture until shortly before his death from heart failure at age 82 in New York City.

See also History of microbiology; History of public health; History of the development of antibiotics

DYSENTERY

Dysentery is an infectious disease that has ravaged armies, refugee camps, and prisoner-of-war camps throughout history. The disease still is a major problem in developing countries with primitive sanitary facilities.

The acute form of dysentery, called shigellosis or bacillary dysentery, is caused by the bacillus (bacterium) of the genus *Shigella,* which is divided into four subgroups and distributed worldwide. Type A, *Shigella dysenteriae,* is a particularly virulent species. Infection begins from the solid waste from someone infected with the bacterium. Contaminated soil or water that gets on the hands of an individual often is conveyed to the mouth, where the person contracts the infection. Flies help to spread the bacillus.

Young children living in primitive conditions of overcrowded populations are especially vulnerable to the disease. Adults, though susceptible, usually will have less severe disease because they have gained a limited resistance. **Immunity** as such is not gained by infection, however, and an infected person can become reinfected by the same species of **Shigella**.

Once the bacterium has gained entrance through the mouth, it travels to the lower intestine (colon) where it penetrates the mucosa (lining) of the intestine. In severe cases, the entire colon may be involved, but usually only the lower half of the colon is involved. The incubation period is one to four days, that is the time from infection until symptoms appear.

Symptoms may be sudden and severe in children. They experience abdominal pain or distension, fever, loss of appetite, nausea, vomiting, and diarrhea. Blood and pus will appear in the stool, and the child may pass 20 or more bowel movements a day. Left untreated, he will become dehydrated from loss of water and will lose weight rapidly. If untreated, death may occur within 12 days of infection. If treated or if the

Dysentery epidemic amongst Hutu refugees.

infection is weathered, the symptoms will subside within approximately two weeks.

Adults experience a less severe course of disease. They will initially feel a griping pain in the abdomen, develop diarrhea, though without any blood in the stool at first. Blood and pus will appear soon, however, as episodes of diarrhea recur with increasing frequency. Dysentery usually ends in the adult within four to eight days in mild cases, and up to six weeks in severe infections.

Shigella dysenteriae brings about a particularly virulent infection that can be fatal within 12 to 24 hours. The patient has little or no diarrhea, but experiences delirium, convulsions, and lapses into a coma. Fortunately, infection with this species is uncommon.

Treatment of the patient with dysentery usually is by fluid therapy to replace the liquid and electrolytes lost in sweating and diarrhea. **Antibiotics** may be used, but some Shigella species have developed resistance to them, and in these cases, antibiotics may be relatively ineffective.

Some individuals harbor the bacterium without having symptoms. Like those who are convalescent from the disease, the carriers without symptoms can spread the disease. This may occur by someone with improperly washed hands preparing food, which becomes infected with the organism.

Another form of dysentery called **amebic dysentery** or intestinal amebiasis is spread by a protozoan, *Entamoeba histolytica*. The protozoan occurs in an active form, which infects the bowel, and an encysted form, which forms the source of infection. If the patient develops diarrhea, the active form of amoeba will pass from the bowel and rapidly die. If no diarrhea is present, the amoeba will form a hard cyst about itself and pass from the bowel to be picked up by another victim. Once ingested, it will lose its shell and begin the infectious cycle. Amebic dysentery can be waterborne, so anyone drinking infested water that is not purified is susceptible to infection.

Amebic dysentery is common in the tropics and relatively rare in temperate climates. Infection may be so subtle as to be practically unnoticed. Intermittent bouts of diarrhea, abdominal pain, flatulence, and cramping mark the onset of infection. Spread of infection may occur with the organisms entering the liver, so abdominal tenderness may occur over the area of the liver. Because the amoeba invades the lining of the colon, some bleeding may occur, and in severe infections, the patient may require blood transfusions to replace lost blood.

Treatment, again, is aimed at replacement of lost fluids and the relief of symptoms. Microscopic examination of the stool will reveal the active protozoan or its cysts. Special med-

ications aimed at eradicating the infectious organism may be needed.

An outbreak of amebic dysentery can occur seemingly mysteriously because the carrier of the amoeba may be without symptoms, especially in a temperate zone. A person with inadequate sanitation can spread the disease through food that he has handled. Often, health officials can trace a disease outbreak back to a single kitchen and then test the cooks for evidence of amebic dysentery.

Before the idea of the spread of infectious agents was understood, dysentery often was responsible for more casualties among the ranks of armies than was actual combat. It also was a constant presence among prisoners, who often died because little or no medical assistance was available to them. Dysentery remains a condition present throughout the world that requires vigilance. Prevention is the most effective means to maintain the health of populations living in close quarters. Hand washing, especially among food preparation personnel, and water purification are the most effective means of prevention.

See also Waste water treatment; Water pollution and purification; Water quality

E

E. COLI O157:H7 INFECTION

Escherichia coli, commonly shortened to *E. coli*, is a Gram-negative bacterium that lives in the intestinal tract of humans and other warm-blooded animals. There are many sub-types, or strains of the organism. One strain is designated as O157:H7, based on two antigens that are present on the surface of the bacterium and of the locomotive appendage called the flagella.

In contrast to many of the other strains, *E. coli* O157:H7 is not a normal resident of the humans intestinal tract. When present in the intestinal tract, via the ingestion of contaminated food or water, O157:H7 causes a severe, even life-threatening malady known as hemorrhagic colitis.

E. coli O157:H7 is a strain of enterohemorrhagic *E. coli* that was initially isolated in Argentina in 1977. The strain is thought to have arisen from a genetic **recombination** between another *E. coli* strain and a toxin-producing strain of *Shigella dysenterae* in the intestinal tract of someone. The resulting genetically altered *E. coli* now carried the genetic information for the toxins.

Strain O157:H7 was recognized as a cause of illness in 1982. Then, an outbreak of severe diarrhea was microbiologically traced to a batch of undercooked hamburgers. Most cases are stilled associated with improperly cooked contaminated meat. For this reason, the infection has acquired the cache of "hamburger disease." However, numerous other foods can deliver the **bacteria**, including alfalfa sprouts, unpasteurized fruits juices such as apple juice, lettuce and cheese curds, and raw milk. **Contamination** of vegetables can occur when they are sprayed in the filed with sewage-containing water and then inadequately washed prior to eating. For example, organically grown produce might not be adequately washed, given the perception that the absence of **antibiotics** negates the need for washing.

Meat can become contaminated with fecal material during slaughter. The bacteria are subsequently distributed throughout the meat when the meat is ground. Thorough cooking is necessary to kill the bacteria buried in the ground meat. Although not clear yet, indications are that the ingestion of as few as 10 surviving bacteria can be sufficient to trigger the infection.

E. coli O157:H7 is also passed onto humans via water that has been contaminated with fecal material, typically from cattle who are a reservoir of the bacterium. For example, the contamination of the water supply of Walkerton, Ontario, Canada, by run-off from a neighbouring cattle farm in the summer of 2000 caused thousands of illnesses and killed seven people.

The damage of the infection results from two potent toxins produced by the bacteria. The toxins are known as verotoxin and shiga-like toxin. These toxins are very similar in structure and action as those produced by another bacteria of health concern, *Shigella dysenteriae*, the agent of bacterial **dysentery**. The toxins exert their effect by both physically damaging the host epithelial cell and by preventing repair of the damage, because of the shutdown of the host cell's ability to manufacture new protein. The toxins bind to a specific receptor called Gb3, which is found on the surface of epithelial cells in blood vessels, smooth muscle cells, kidney cells, and red blood cells. The bound toxins inhibit **protein synthesis**, thus killing the cells.

The toxic damage occurs following the tight association of the bacteria with the surface of the intestinal epithelial cells. Research has proven that this association relies on the manufacture and extrusion of a specific protein by the bacteria that acts as an anchor to which the bacteria bind. As binding occurs, the host cells change their configuration, becoming so-called pedestals on which each bacterium sits. At this point the binding of the bacteria with the host cells is tenacious and the infection is established.

Hemorrhagic colitis begins as a severe abdominal pain accompanied by watery diarrhea. As damage to the epithelial cells lining the intestinal tract occurs, the diarrhea becomes bloody. Vomiting can also occur. These symptoms, as severe and debilitating as they are, usually last only between one and

two weeks, and terminate naturally as the body's immune defenses successfully cope with the infection. Usually no permanent damage results from the infection. However in those who are immunocompromised and in children, the disease can become more disseminated. Damage to the kidney can be so devastating that complete loss of kidney function occurs. If not treated the death rate from hemolytic anemia is high. Even with rapid diagnoses and treatment that includes antibiotics, blood transfusions and kidney dialysis, the death rate is still three to five percent.

Approximately ten to fifteen per cent of those infected with strain O157:H7 develop hemolytic anemia. The syndrome is the leading cause of sudden-onset kidney failure in children in the world. As well, the elderly can develop a condition known as thrombocytopenic purpura, which consists of a fever and nerve damage. In the elderly, this malady can kill almost half of those who become infected.

The chances of infection from *E. coli* O157:H7 are greatly lessened by proper food preparation, washing of food surfaces that have been exposed to raw ground meat, and proper personal **hygiene**, especially hand washing. Also, since the bacterium is very sensitive to heat, boiling suspect water prior to drinking the water is a sure way to eliminate the risk of infection from the bacteria and to inactivate the toxins.

Also, a **vaccine** for cattle is in the final testing stages prior to being approved for sale. Approval is expected in 2002. The vaccine blocks the binding and pedestal formation by the bacteria in cattle. The bacteria remain free in the intestinal tract and so are washed out of the cow. Eliminating the reservoir of the organism lessens the spread of O157:H7 infection to humans.

See also Anti-adhesion mechanisms; *Escherichia coli*; Food safety; Vaccination

EAR INFECTIONS, CHRONIC

Chronic ear infection, which is also referred to as chronic otitis media, is a recurring infection of the middle ear that occurs in animals and in humans. In humans, children between a few months of age and about six years of age are the most susceptible. The infection can be caused by **bacteria** and, occasionally, by **viruses**.

The ear consists of outer, middle, and inner regions. The outer ear is the visible portion that channels sound vibrations to the middle ear. The middle ear contains three small bones that pass on the vibration to the nerve endings housed in the inner ear. The middle ear is connected to the nasal cavity and the throat by a drainage tube known as the Eustachian tube. Improper drainage from the Eustachian tubes result in a retention of fluid in the middle ear, which can become infected by bacteria.

Such infections are common in children. Each year in the United States, over 10 million children are treated for ear infections. However, ear infections tend to be infrequent and disappear as the construction of the ear changes with age. Specifically, the Eustachian tube becomes more slanted in orientation, which promotes drainage that is more efficient. However, in some children the normally short-term (or acute) middle ear infections begin to recur. For these children, many ear infections can occur in the first six or so years of life. Chronic ear infections affects about two out of every 10,000 people.

In some cases, surgical intervention is necessary to install a plastic drainage tube (a procedure called myringotomy) or to remove infected adenoids or tonsils, which can swell and block the eustachian tube. Myringotomy is one of the most common operations that are performed in the United States. As the ear matures structurally and the eustachian tube acquires the ability to drain more freely, the tube is removed.

As with other chronic bacterial infections, the symptoms associated with chronic ear infections can be less severe and uncomfortable than those of the acute form of the infection. Chronic infections may thus escape detection for long periods of time.

Usually a chronic ear infection is more inconvenient and uncomfortable than a health threat. However, in some cases, the chronic bacterial or viral ear infections can lead to complications that are much more serious. The infection can spread into the bones of the ear. Also, the increased pressure from the build-up of fluid can rupture the eardrum. Such damage can produce permanent impairment of hearing.

Another damaging aspect of chronic ear infections, which is shared with other chronic bacterial infections, is the damage to tissues that results from a prolonged immune response to the infection. The failure to clear the infection can produce a prolonged immune response. This response, particularly **inflammation**, can be damaging to tissue.

Treatment consists of decongestants or antihistamines to promote drainage through the Eustachian tube, and of **antibiotics** in the case of a **bacterial infection**. Even with treatment a chronic infection can take weeks or months to completely clear. Adherence to the treatment schedule is critical, especially since the symptoms of chronic ear infections can pass before the infection is fully cleared. Stopping therapy when the symptoms fade may allow the bacteria that are still surviving to become re-established as another infection. Moreover, because the bacteria were exposed to an antibacterial agent, resistance to that agent can develop, making the recurrent infection harder to eradicate.

See also Bacteria and bacterial infection

EBOLA VIRUS

The Ebola virus is one of two members of a family of **viruses** that is designated as the Filoviridae. The name of the virus comes from a river located in the Democratic Republic of the Congo, where the virus was discovered.

The species of Ebola virus are among a number of viruses that cause a disease that is typified by copious internal bleeding and bleeding from various orifices of the body, including the eyes. The disease can be swiftly devastating and results in death in over 90% of cases.

To date, four species of Ebola virus have been identified, based on differences in their genetic sequences and in the immun reaction they elicit in infected individuals. Three of the species cause disease in humans. These are Ebola-Zaire (isolated in 1976), Ebola-Sudan (also isolated in 1976), and Ebola–Ivory Coast (isolated in 1994). The fourth species, called Ebola-Reston, causes disease in primates. The latter species is capable of infecting humans but so far has not caused disease in humans. Ebola-Reston is named for the United States military primate research facility where the virus was isolated, during a 1989 outbreak of the disease caused by infected monkeys that had been imported from the Philippines. Until the non-human involvement of the disease was proven, the outbreak was thought to be the first outside of Africa.

The appearance of the Ebola virus only dates back to 1976. The explosive onset of the illness and the under-developed and wild nature of the African region of the virus's appearance, has complicated the definitive determinations of the origin and natural habitat of Ebola. The source of the Ebola virus is still unknown. However, given that filovirus, which produce similar effects, establish a latent infection in African monkeys, macaques, and chimpanzees, scientists consider the possibility that the Ebola virus likewise normally resides in an animal that lives in Africa. A search for Ebola virus in such primates has so far not revealed evidence of the virus.

Almost all confirmed cases of Ebola from 1976 to 2002 have been in Africa. In the latest outbreak, which has been ongoing since late in 2001, 54 people have died in the Gabon as of February of 2002. In the past, one individual in Liberia presented immunological evidence of exposure to Ebola, but had no symptoms. As well, a laboratory worker in England developed Ebola fever as a result of a laboratory accident in which the worker was punctured by an Ebola-containing needle.

The Ebola virus produces a high fever, headache, muscle aches, abdominal pain, tiredness and diarrhea within a few days after infecting a person. Some people will also display bloody diarrhea and vomit blood. At this stage of the disease some people recover. But, for most of those who are infected, the disease progresses within days to produce copious internal bleeding, shock and death.

Outbreaks of infection with the Ebola virus appear sporadically and suddenly. The outbreak rapidly moves through the local population and often just as quickly ends. The initial infection is presumable by contact between the person and the animal that harbors the virus. Subsequent person-to-person spread likely occurs by **contamination** with the infected blood or body tissues of an infected person in the home or hospital setting, or via contaminated needles. The fact that infected people tend to be in more under-developed regions, where even the health care facilities are not as likely to be equipped with isolation wards, furthers the risk of spread. The person-to-person passage is immediate; unlike the animal host, people do not harbor the virus for lengthy periods of time.

The possibility of air-borne transmission of the virus is debatable. Ebola-Reston may well have been transmitted from monkey to monkey in the Reston military facility via the air distribution system, since some of the monkeys that were infected were never in physical contact with the other infected monkeys. However, if the other species of the virus are capable of similar transmission, this has not yet been documented. Laboratory studies have shown that Ebola virus can remain infectious when aerosolized. But the current consensus is that airborne transmission is possible but plays a minor role in the spread of the virus.

Negative stain electron micrograph of an Ebola virus.

In the intervening years between the sporadic outbreaks, the Ebola virus probably is resident in the natural reservoir.

Currently there is no cure for the infection caused by the Ebola virus. However, near the end of an outbreak of the virus in 1995 in Kikwit, Africa, blood products from survivors of the infection were transfused into those actively experiencing the disease. Of those eight people who received the blood, only one person died. Whether or not the transfused blood conveyed protective factor was not ascertained. A detailed examination of this possibility awaits another outbreak.

The molecular basis for the establishment of an infection by the Ebola virus is still also more in the realm of proposal than fact. One clue has been the finding of a glycoprotein that is a shortened version of the viral constituent in the in the circulating fluid of humans and monkeys. This protein has been suggested to function as a decoy for the **immune system**, diverting the immune defenses from the actual site of viral infection. Another immunosuppressive mechanism may be the selective invasion and damage of the spleen and the lymph nodes, which are vital in the functioning of the immune system.

The devastating infection caused by the Ebola virus is all the more remarkable given the very small size of the viral genome, or **complement** of genetic material. Fewer than a dozen genes have been detected. How the virus establishes an infection and evades the host immune system with only the capacity to code for less than twelve proteins is unknown.

See also Hemorrhagic fevers and diseases; Zoonoses

ECOLOGY OF THE ORAL CAVITY • *see* MICROBIAL FLORA OF THE ORAL CAVITY, DENTAL CARIES

ECOLOGY OF THE STOMACH AND GASTROINTESTINAL TRACT • *see* MICROBIAL FLORA OF THE STOMACH AND GASTROINTESTINAL TRACT

ECONOMIC USES AND BENEFITS OF MICROORGANISMS

Microorganisms have been used as tools for the production of products for millennia. Even in ancient times, the ability to produce vinegar by allowing water to percolate through wood shavings was known and widely practiced. Likewise, the **transformation** of a **yeast** suspension into beer or a suspension of crushed grapes into wine was common knowledge. The basis of these events may not have been known, but that did not impede the sale or trade of such products.

These economic uses of microorganisms are the earliest examples of **biotechnology**. As the knowledge of **bacteria** and yeast-chemical behaviors grew, other biotechnological uses for the microbes were found. A few examples include the use of the bacterium *Lactobacillus acidophilus* to produce yogurt, the exploitation of a number of different bacteria to produce a variety of cheeses, and the **fermentation** of cabbage to produce sauerkraut. In the agricultural sector, the discovery of the ability of *Rhizobium spp.* to convert elemental nitrogen to a form that was useable by a growing plant, led to the use of the microorganism as a living fertilizer that grew in association with the plant species.

In more modern times, the use of microorganisms as biotechnological agents of profit has not only continued but has explosively increased. Indeed the biotechnology sector as it is recognized today, is already a multi-billion dollar sector worldwide.

The unraveling of the structure of **DNA (deoxyribonucleic acid)**, various species of **ribonucleic acid** (**RNA**), and the various processes whereby the manufacture of protein from the nucleic acid templates occurs was pivotal in advancing the use of microorganisms as factories. As important was the discovery of how to remove DNA from one region of the genome and move the DNA in a controlled way to another region of the same DNA, or DNA in a completely different organism (prokaryotic or eukaryotic). These **gene** splicing technologies, which can be accomplished by various splicing and reannealing **enzymes**, or by the use of **viruses** or mobile regions of viral DNA (such as **transposons**) as vectors have allowed biotechnologists to create what are termed "designer genes," which are designed for a specific purpose. This ability has fueled the use of microorganisms for economic gain and/or benefit.

The gene for the production of human insulin has been transferred into the genome of the common intestinal tract bacterium ***Escherichia coli***. Successful expression and excretion of human insulin by the bacteria allows the production of a large amount of insulin. Additionally, because the insulin is identical to that produced in a human being, the chance of immune reaction against the protein is virtually nonexistent. The example of insulin reflects both the health benefit of the use of microbes and the economic benefit to be realized, since the mass production of insulin that is possible using bacteria lowers the cost of the product.

Dispensing beer into kegs.

Other medical uses of microorganisms, particularly in the production of **antibiotics**, have been the greatest boon to humans and other animals. The list of maladies that can now be treated using microbiologically derived compounds is lengthy, and includes cystic fibrosis, hemophilia, **hepatitis** B, Karposi's sarcoma, rejection of transplanted organs, growth hormone deficiency, and cancer. The worldwide sales of medical and pharmaceutical drugs of microbial origin now exceeds U.S. $13 billion annually.

Microorganisms have also been harnessed as factories to produce compounds that are used in areas as divers as textile manufacture, agriculture, and nutrition. Enzymes discovered in bacteria that can exist at very elevated temperatures (thermophilic, or "heat loving" bacteria) cab be used to age denim to produce a "pre-washed" look. Similar enzymes are being exploited in laundry detergent that operates in hot water.

Microorganisms are used to enhance the nutritional content of plants and other food sources. The growing nutraceutical sector relies in part on the nutritional enhancements afforded by microbes. Bacteria are also useful in providing a degree of resistance to plants. An example is the use of *Bacillus thuringensis* to supply a protein that is lethal to insect when they consume it. The use of bacterial insecticides has reduced the use of chemical insecticides, which is both a cost savings to the producer and less stressful on the environment. Other bacterial enzymes and constituents of the organisms are utilized to produce materials such as plastic.

A process known as DNA fingerprinting, which relies upon enzymes that are produced and operate in bacteria, has enabled the tracing of the fate of genes in plant and animal populations, and enhanced gathering of evidence at crime scenes.

The mode of growth of bacterial populations has also proved to be exploitable as a production tool. A prime example is the surface-adherent mode of **bacterial growth** that is termed a **biofilm**. Although not known at the time, the production of vinegar hundreds of years ago was, as now, based on the percolation of water through biofilms growing on wood shavings. Immobilized bacteria can produce all manner of compounds. As well, the cells can provide a physical barrier to the flow of fluid. This dynamic aspect has been utilized in a so far small-scale way to increase the production of oil from fields oil thought to be depleted. Bacteria can plug up the zones were water and oil flows most easily. Subsequent pumping of water through the field forces the oil still resident in lower permeability areas to the surface.

With the passing of time, the realized and potential benefits of microorganisms and the implementation of strict standards of microbe use, is lessening the concern over the use of engineered microorganisms for economic and social benefit. The use of microorganisms can only increase.

See also Bioremediation; Composting, microbiological aspects; DNA chips and micro arrays

EDELMAN, GERALD M. (1929-)

American biochemist

For his "discoveries concerning the chemical structure of antibodies," Gerald M. Edelman and his associate Rodney Porter received the 1972 Nobel Prize in physiology or medicine. During a lecture Edelman gave upon acceptance of the prize, he stated that **immunology** "provokes unusual ideas, some of which are not easily come upon through other fields of study.... For this reason, immunology will have a great impact on other branches of biology and medicine." He was to prove his own prediction correct by using his discoveries to draw conclusions not only about the **immune system** but about the nature of consciousness as well.

Born in New York City to Edward Edelman, a physician, and Anna Freedman Edelman, Gerald Maurice Edelman attended New York City public schools through high school. After graduating, he entered Ursinus College, in Collegeville, Pennsylvania, where he received his B.S. in chemistry in 1950. Four years later, he earned an M.D. degree from the University of Pennsylvania's Medical School, spending a year as medical house officer at Massachusetts General Hospital.

In 1955, Edelman joined the United States Army Medical Corps, practicing general medicine while stationed at a hospital in Paris. There, Edelman benefited from the heady atmosphere surrounding the Sorbonne, where future Nobel laureates **Jacques Lucien Monod** and **François Jacob** were originating a new study, **molecular biology**. Following his 1957 discharge from the Army, Edelman returned to New York City to take a position at Rockefeller University studying under Henry Kunkel. Kunkel, with whom Edelman would conduct his Ph.D. research, and who was examining the unique flexibility of antibodies at the time.

Antibodies are produced in response to infection in order to work against diseases in diverse ways. They form a class of large blood proteins called globulins—more specifically, immunoglobulins—made in the body's lymph tissues. Each immunoglobulin is specifically directed to recognize and incapacitate one **antigen**, the chemical signal of an infection. Yet they all share a very similar structure.

Through the 1960s and 1970s, a debate raged between two schools of scientists to explain the situation whereby antibodies share so many characteristics yet are able to perform many different functions. In one camp, George Wells Beadle and **Edward Lawrie Tatum** argued that despite the remarkable diversity displayed by each **antibody**, each immunoglobulin, must be coded for by a single **gene**. This has been referred to as the "one gene, one protein" theory. But, argued the opposing camp, led by the Australian physician Sir **Frank Macfarlane Burnet**, if each antibody required its own code within the **DNA** (**deoxyribonucleic acid**), the body's master plan of protein structure, the immune system alone would take up all the possible codes offered by the human DNA.

Both camps generated theories, but Edelman eventually disagreed with both sides of the debate, offering a third possibility for antibody synthesis in 1967. Though not recognized at the time because of its radical nature, the theory he and his associate, Joseph Gally, proposed would later be confirmed as essentially correct. It depended on the vast diversity that can come from chance in a system as complex as the living organism. Each time a cell divided, they theorized, tiny errors in the transcription—or reading of the code—could occur, yielding slightly different proteins upon each misreading. Edelman and Gally proposed that the human body turns the advantage of this variability in **immunoglobulins** to its own ends. Many strains of antigens when introduced into the body modify the shape of the various immunoglobulins in order to prevent the recurrence of disease. This is why many illnesses provide for their own cure—why humans can only get chicken pox once, for instance.

But the proof of their theory would require advances in the state of biochemical techniques. Research in the 1950s and 1960s was hampered by the difficulty in isolating immunoglobulins. The molecules themselves are comparatively large, too large to be investigated by the chemical means then available. Edelman and Rodney Porter, with whom Edelman was to be honored with the Nobel Prize, sought

methods of breaking immunoglobulins into smaller units that could more profitably be studied. Their hope was that these fragments would retain enough of their properties to provide insight into the functioning of the whole.

Porter became the first to split an immunoglobulin, obtaining an "active fragment" from rabbit blood as early as 1950. Porter believed the immunoglobulin to be one long continuous molecule made up of 1,300 amino acids—the building blocks of proteins. However, Edelman could not accept this conclusion, noting that even insulin, with its 51 amino acids, was made up of two shorter strings of amino acid chains working as a unit. His doctoral thesis investigated several methods of splitting immunoglobulin molecules, and, after receiving his Ph.D. in 1960 he remained at Rockefeller as a faculty member, continuing his research.

Porter's method of splitting the molecules used **enzymes** that acted as chemical knives, breaking apart amino acids. In 1961 Edelman and his colleague, M. D. Poulik succeeded in splitting IgG—one of the most studied varieties of immunoglobulin in the blood—into two components by using a method known as "reductive cleavage." The technique allowed them to divide IgG into what are known as light and heavy chains. Data from their experiments and from those of the Czech researcher, Frantisek Franek, established the intricate nature of the antibody's "active sight." The sight occurs at the folding of the two chains, which forms a unique pocket to trap the antigen. Porter combined these findings with his, and, in 1962, announced that the basic structure of IgG had been determined. Their experiments set off a flurry of research into the nature of antibodies in the 1960s. Information was shared throughout the scientific community in a series of informal meetings referred to as "Antibody Workshops," taking place across the globe. Edelman and Porter dominated the discussions, and their work led the way to a wave of discoveries.

Still, a key drawback to research remained. In any naturally obtained immunoglobulin sample a mixture of ever so slightly different molecules would reduce the overall purity. Based on a crucial finding by Kunkel in the 1950s, Porter and Edelman concentrated their study on myelomas, cancers of the immunoglobulin-producing cells, exploiting the unique nature of these cancers. Kunkel had determined that since all the cells produced by these cancerous myelomas were descended from a common ancestor they would produce a homogeneous series of antibodies. A pure sample could be isolated for experimentation. Porter and Edelman studied the amino acid sequence in subsections of different myelomas, and in 1965, as Edelman would later describe it: "Mad as we were, [we] started on the whole molecule." The project, completed in 1969, determined the order of all 1,300 amino acids present in the protein, the longest sequence determined at that time.

Throughout the 1970s, Edelman continued his research, expanding it to include other substances that stimulate the immune system, but by the end of the decade the principle he and Poulik uncovered led him to conceive a radical theory of how the brain works. Just as the structurally limited immune system must deal with myriad invading organisms, the brain must process vastly complex sensory data with a theoretically limited number of switches, or neurons.

Rather than an incoming sensory signal triggering a predetermined pathway through the nervous system, Edelman theorized that it leads to a **selection** from among several choices. That is, rather than seeing the nervous system as a relatively fixed biological structure, Edelman envisioned it as a fluid system based on three interrelated stages of functioning.

In the formation of the nervous system, cells receiving signals from others surrounding them fan out like spreading ivy—not to predetermined locations, but rather to regions determined by the concert of these local signals. The signals regulate the ultimate position of each cell by controlling the production of a cellular glue in the form of cell-adhesion molecules. They anchor neighboring groups of cells together. Once established, these cellular connections are fixed, but the exact pattern is different for each individual.

The second feature of Edelman's theory allows for an individual response to any incoming signal. A specific pattern of neurons must be made to recognize the face of one's grandmother, for instance, but the pattern is different in every brain. While the vast complexity of these connections allows for some of the variability in the brain, it is in the third feature of the theory that Edelman made the connection to immunology. The neural networks are linked to each other in layers. An incoming signal passes through and between these sheets in a specific pathway. The pathway, in this theory, ultimately determines what the brain experiences, but just as the immune system modifies itself with each new incoming virus, Edelman theorized that the brain modifies itself in response to each new incoming signal. In this way, Edelman sees all the systems of the body being guided in one unified process, a process that depends on organization but that accommodates the world's natural randomness.

Dr. Edelman has received honorary degrees from a number of universities, including the University of Pennsylvania, Ursinus College, Williams College, and others. Besides his Nobel Prize, his other academic awards include the Spenser Morris Award, the Eli Lilly Prize of the American Chemical Society, Albert Einstein Commemorative Award, California Institute of Technology's Buchman Memorial Award, and the Rabbi Shai Schaknai Memorial Prize.

A member of many academic organizations, including New York and National Academy of Sciences, American Society of Cell Biologists, Genetics Society, American Academy of Arts and Sciences, and the American Philosophical Society, Dr. Edelman is also one of the few international members of the Academy of Sciences, Institute of France. In 1974, he became a Vincent Astor Distinguished Professor, serving on the board of governors of the Weizmann Institute of Science and is also a trustee of the Salk Institute for Biological Studies. Dr. Edelman married Maxine Morrison on June 11, 1950; the couple have two sons and one daughter.

See also Antibody and antigen; Antibody formation and kinetics; Antibody, monoclonal; Antibody-antigen, biochemical and molecular reactions; Antigenic mimicry

EHRLICH, PAUL (1854-1915)

German physician

Paul Ehrlich's pioneering experiments with cells and body tissue revealed the fundamental principles of the **immune system** and established the legitimacy of chemotherapy—the use of chemical drugs to treat disease. His discovery of a drug that cured **syphilis** saved many lives and demonstrated the potential of systematic drug research. Ehrlich's studies of dye reactions in blood cells helped establish hematology, the scientific field concerned with blood and blood-forming organs, as a recognized discipline. Many of the new terms he coined as a way to describe his innovative research, including "chemotherapy," are still in use. From 1877 to 1914, Ehrlich published 232 papers and books, won numerous awards, and received five honorary degrees. In 1908, Ehrlich received the Nobel Prize in medicine or physiology.

Ehrlich was born on March 14, 1854, in Strehlen, Silesia, once a part of Germany, but now a part of Poland known as Strzelin. He was the fourth child after three sisters in a Jewish family. His father, Ismar Ehrlich, and mother, Rosa Weigert, were both innkeepers. As a boy, Ehrlich was influenced by several relatives who studied science. His paternal grandfather, Heimann Ehrlich, made a living as a liquor merchant but kept a private laboratory and gave lectures on science to the citizens of Strehlen. Karl Weigert, cousin of Ehrlich's mother, became a well-known pathologist. Ehrlich, who was close friends with Weigert, often joined his cousin in his lab, where he learned how to stain cells with dye in order to see them better under the **microscope**. Ehrlich's research into the dye reactions of cells continued during his time as a university student. He studied science and medicine at the universities of Breslau, Strasbourg, Freiburg, and Leipzig. Although Ehrlich conducted most of his course work at Breslau, he submitted his final dissertation to the University of Leipzig, which awarded him a medical degree in 1878.

Ehrlich's 1878 doctoral thesis, "Contributions to the Theory and Practice of Histological Staining," suggests that even at this early stage in his career he recognized the depth of possibility and discovery in his chosen research field. In his experiments with many dyes, Ehrlich had learned how to manipulate chemicals in order to obtain specific effects: Methylene blue dye, for example, stained nerve cells without discoloring the tissue around them. These experiments with dye reactions formed the backbone of Ehrlich's career and led to two important contributions to science. First, improvements in staining permitted scientists to examine cells, healthy or unhealthy, and **microorganisms**, including those that caused disease. Ehrlich's work ushered in a new era of medical diagnosis and histology (the study of cells), which alone would have guaranteed Ehrlich a place in scientific history. Secondly, and more significantly from a scientific standpoint, Ehrlich's early experiments revealed that certain cells have an affinity to certain dyes. To Ehrlich, it was clear that chemical and physical reactions were taking place in the stained tissue. He theorized that chemical reactions governed all biological life processes. If this were true, Ehrlich reasoned, then chemicals could perhaps be used to heal diseased cells and to attack harmful microorganisms. Ehrlich began studying the chemical structure of the dyes he used and postulated theories for what chemical reactions might be taking place in the body in the presence of dyes and other chemical agents. These efforts would eventually lead Ehrlich to study the immune system.

Upon Ehrlich's graduation, medical clinic director Friedrich von Frerichs immediately offered the young scientist a position as head physician at the Charite Hospital in Berlin. Von Frerichs recognized that Ehrlich, with his penchant for strong cigars and mineral water, was a unique talent, one that should be excused from clinical work and be allowed to pursue his research uninterrupted. The late nineteenth century was a time when infectious diseases like cholera and **typhoid fever** were incurable and fatal. Syphilis, a sexually transmitted disease caused by a then unidentified microorganism, was an epidemic, as was **tuberculosis**, another disease whose cause had yet to be named. To treat human disease, medical scientists knew they needed a better understanding of harmful microorganisms.

At the Charite Hospital, Ehrlich studied blood cells under the microscope. Although blood cells can be found in a perplexing multiplicity of forms, Ehrlich was with his dyes able to begin identifying them. His systematic cataloging of the cells laid the groundwork for what would become the field of hematology. Ehrlich also furthered his understanding of chemistry by meeting with professionals from the chemical industry. These contacts gave him information about the structure and preparation of new chemicals and kept him supplied with new dyes and chemicals.

Ehrlich's slow and steady work with stains resulted in a sudden and spectacular achievement. On March 24, 1882, Ehrlich had heard **Robert Koch** announce to the Berlin Physiological Society that he had identified the bacillus causing tuberculosis under the microscope. Koch's method of staining the bacillus for study, however, was less than ideal. Ehrlich immediately began experimenting and was soon able to show Koch an improved method of staining the tubercle bacillus. The technique has since remained in use.

On April 14, 1883, Ehrlich married 19-year-old Hedwig Pinkus in the Neustadt Synagogue. Ehrlich had met Pinkus, the daughter of an affluent textile manufacturer of Neustadt, while visiting relatives in Berlin. The marriage brought two daughters. In March, 1885, von Frerichs committed suicide and Ehrlich suddenly found himself without a mentor. Von Frerichs's successor as director of Charite Hospital, Karl Gerhardt, was far less impressed with Ehrlich and forced him to focus on clinical work rather than research. Though complying, Ehrlich was highly dissatisfied with the change. Two years later, Ehrlich resigned from the Charite Hospital, ostensibly because he wished to relocate to a dry climate to cure himself of tuberculosis. The mild case of the disease, which Ehrlich had diagnosed using his staining techniques, was almost certainly contracted from cultures in his lab. In September of 1888, Ehrlich and his wife embarked on an extended journey to southern Europe and Egypt and returned to Berlin in the spring of 1889 with Ehrlich's health improved.

In Berlin, Ehrlich set up a small private laboratory with financial help from his father-in-law, and in 1890, he was hon-

ored with an appointment as Extraordinary Professor at the University of Berlin. In 1891, Ehrlich accepted Robert Koch's invitation to join him at the Institute for Infectious Diseases, newly created for Koch by the Prussian government. At the institute, Koch began his immunological research by demonstrating that mice fed or injected with the toxins ricin and abrin developed antitoxins. He also proved that antibodies were passed from mother to offspring through breast milk. Ehrlich joined forces with Koch and **Emil Adolf von Behring** to find a cure for **diphtheria**, a deadly childhood disease. Although von Behring had identified the antibodies to diphtheria, he still faced great difficulties transforming the discovery into a potent yet safe cure for humans. Using blood drawn from horses and goats infected with the disease, the scientists worked together to concentrate and purify an effective antitoxin. Ehrlich's particular contribution to the cure was his method of measuring an effective dose.

The commercialization of a diphtheria antitoxin began in 1892 and was manufactured by Höchst Chemical Works. Royalties from the drug profits promised to make Ehrlich and von Behring wealthy men. But Ehrlich, possibly at von Behring's urging, accepted a government position in 1885 to monitor the production of the diphtheria serum. Conflict-of-interest clauses obligated Ehrlich to withdraw from his profit-sharing agreement. Forced to stand by as the diphtheria antitoxin made von Behring a wealthy man, he and von Behring quarreled and eventually parted. Although it is unclear whether bitterness over the royalty agreement sparked the quarrel, it certainly couldn't have helped a relationship that was often tumultuous. Although the two scientists continued to exchange news in letters, both scientific and personal, the two scientists never met again.

In June of 1896, the Prussian government invited Ehrlich to direct its newly created Royal Institute for Serum Research and Testing in Steglitz, a suburb of Berlin. For the first time, Ehrlich had his own institute. In 1896, Ehrlich was invited by Franz Adickes, the mayor of Frankfurt, and by Friedrich Althoff, the Prussian Minister of Educational and Medical Affairs, to move his research to Frankfurt. Ehrlich accepted and the Royal Institute for Experimental Therapy opened on November 8, 1899. Ehrlich was to remain as its director until his death sixteen years later. The years in Frankfurt would prove to be among Ehrlich's most productive.

In his speech at the opening of the Institute for Experimental Therapy, Ehrlich seized the opportunity to describe in detail his "side-chain theory" of how antibodies worked. "Side-chain" is the name given to the appendages on benzene molecules that allow it to react with other chemicals. Ehrlich believed all molecules had similar side-chains that allowed them to link with molecules, nutrients, infectious toxins and other substances. Although Ehrlich's theory is false, his efforts to prove it led to a host of new discoveries and guided much of his future research.

The move to Frankfurt marked the dawn of **chemotherapy** as Ehrlich erected various chemical agents against a host of dangerous microorganisms. In 1903, scientists had discovered that the cause of **sleeping sickness**, a deadly disease prevalent in Africa, was a species of trypanosomes (parasitic protozoans). With help from Japanese scientist Kiyoshi Shiga, Ehrlich worked to find a dye that destroyed trypanosomes in infected mice. In 1904, he discovered such a dye, which was dubbed "trypan red."

Success with trypan red spurred Ehrlich to begin testing other chemicals against disease. To conduct his methodical and painstaking experiments with an enormous range of chemicals, Ehrlich relied heavily on his assistants. To direct their work, he made up a series of instructions on colored cards in the evening and handed them out each morning. Although such a management strategy did not endear him to his lab associates, and did not allow them opportunity for their own research, Ehrlich's approach was often successful. In one famous instance, Ehrlich ordered his staff to disregard the accepted notion of the chemical structure of atoxyl and to instead proceed in their work based on his specifications of the chemical. Two of the three medical scientists working with Ehrlich were appalled at his scientific heresy and ended their employment at the laboratory. Ehrlich's hypothesis concerning atoxyl turned out to have been correct and would eventually lead to the discovery of a chemical cure for syphilis.

In September of 1906, Ehrlich's laboratory became a division of the new Georg Speyer Haus for Chemotherapeutical Research. The research institute, endowed by the wealthy widow of Georg Speyer for the exclusive purpose of continuing Ehrlich's work in chemotherapy, was built next to Ehrlich's existing laboratory. In a speech at the opening of the new institute, Ehrlich used the phrase "magic bullets" to illustrate his hope of finding chemical compounds that would enter the body, attack only the offending microorganisms or malignant cells, and leave healthy tissue untouched. In 1908, Ehrlich's work on **immunity**, particularly his contribution to the diphtheria antitoxin, was honored with the Nobel Prize in medicine or physiology. He shared the prize with Russian bacteriologist **Élie Metchnikoff**.

By the time Ehrlich's lab formally joined the Speyer Haus, he had already tested over 300 chemical compounds against trypanosomes and the syphilis spirochete (distinguished as slender and spirally undulating **bacteria**). With each test given a laboratory number, Ehrlich was testing compounds numbering in the nine hundreds before realizing that "compound 606" was a highly potent drug effective against relapsing fever and syphilis. Due to an assistant's error, the potential of compound 606 had been overlooked for nearly two years until Ehrlich's associate, Sahashiro Hata, experimented with it again. On June 10, 1909, Ehrlich and Hata filed a patent for 606 for its use against relapsing fever.

The first favorable results of 606 against syphilis were announced at the Congress for Internal Medicine held at Wiesbaden in April 1910. Although Ehrlich emphasized he was reporting only preliminary results, news of a cure for the devastating and widespread disease swept through the European and American medical communities and Ehrlich was besieged with requests for the drug. Physicians and victims of the disease clamored at his doors. Ehrlich, painfully aware that mishandled dosages could blind or even kill patients, begged physicians to wait until he could test 606 on

ten or twenty thousand more patients. There was no halting the demand, however, and the Georg Speyer Haus ultimately manufactured and distributed 65,000 units of 606 to physicians all over the globe free of charge. Eventually, the large-scale production of 606, under the commercial name "Salvarsan," was taken over by Höchst Chemical Works. The next four years, although largely triumphant, were also filled with reports of patients' deaths and maiming at the hands of doctors who failed to administer Salvarsan properly.

In 1913, in an address to the International Medical Congress in London, Ehrlich cited trypan red and Salvarsan as examples of the power of chemotherapy and described his vision of chemotherapy's future. The City of Frankfurt honored Ehrlich by renaming the street in front of the Georg Speyer Haus "Paul Ehrlichstrasse." Yet in 1914, Ehrlich was forced to defend himself against claims made by a Frankfurt newspaper, *Die Wahrheit* (The Truth), that Ehrlich was testing Salvarsan on prostitutes against their will, that the drug was a fraud, and that Ehrlich's motivation for promoting it was personal monetary gain. In June 1914, Frankfurt city authorities took action against the newspaper and Ehrlich testified in court as an expert witness. Ehrlich's name was finally cleared and the newspaper's publisher sentenced to a year in jail, but the trial left Ehrlich deeply depressed. In December, 1914, he suffered a mild stroke.

Ehrlich's health failed to improve and the start of World War I had further discouraged him. Afflicted with arteriosclerosis, his health deteriorated rapidly. He died in Bad Homburg, Prussia (now Germany), on August 20, 1915, after a second stroke. Ehrlich was buried in Frankfurt. Following the German Nazi era, during which time Ehrlich's widow and daughters were persecuted as Jews before fleeing the country and the sign marking Paul Ehrlichstrasse was torn down, Frankfurt once again honored its famous resident. The Institute for Experimental Therapy changed its name to the Paul Ehrlich Institute and began offering the biennial Paul Ehrlich Prize in one of Ehrlich's fields of research as a memorial to its founder.

See also History of immunology; History of microbiology; History of public health; History of the development of antibiotics; Infection and resistance

ELECTRON MICROSCOPE, TRANSMISSION AND SCANNING

Described by the Nobel Society as "one of the most important inventions of the century," the electron **microscope** is a valuable and versatile research tool. The first working models were constructed by German engineers **Ernst Ruska** and Max Knoll in 1932, and since that time, the electron microscope has found numerous applications in chemistry, engineering, medicine, **molecular biology** and genetics.

Electron microscopes allow molecular biologists to study small structural details related to cellular function. Using an electron microscope, it is possible to observe and study many internal cellular structures (organelles). Electron microscopy can also be used to visualize proteins, virus particles, and other microbiological materials.

A transmission electron microscope.

At the turn of the twentieth century, the science of microscopy had reached an impasse: because all optical microscopes relied upon visible light, even the most powerful could not detect an image smaller than the wavelength of light used. This was tremendously frustrating for physicists, who were anxious to study the structure of matter on an atomic level. Around this time, French physicist **Louis de Broglie** theorized that subatomic particles sometimes act like waves, but with much shorter wavelengths. Ruska, then a student at the University of Berlin, wondered why a microscope couldn't be designed that was similar in function to a normal microscope but used a beam of electrons instead of a beam of light. Such a microscope could resolve images thousands of times smaller than the wavelength of visible light.

There was one major obstacle to Ruska's plan, however. In a compound microscope, a series of lenses are used to focus, magnify, and refocus the image. In order for an electron-based instrument to perform as a microscope, some device was required to focus the electron beam. Ruska knew that electrons could be manipulated within a magnetic field, and in the late 1920s, he designed a magnetic coil that acted as an electron lens. With this breakthrough, Ruska and Knoll constructed their first electron microscope. Though the prototype model was capable of magnification of only a few hundred power (about that of an average laboratory microscope), it proved that electrons could indeed be used in microscopy.

The microscope built by Ruska and Knoll is similar in principle to a compound microscope. A beam of electrons is directed at a specimen sliced thin enough to allow the beam to pass through. As they travel through, the electrons are deflected according to the atomic structure of the specimen. The beam is then focused by the magnetic coil onto a photographic plate; when developed, the image on the plate shows the specimen at very high magnification.

Scientists worldwide immediately embraced Ruska's invention as a major breakthrough in microscopy, and they directed their own efforts toward improving upon its precision and flexibility. A Canadian-American physicist, James Hillier, constructed a microscope from Ruska's design that was nearly 20 times more powerful. In 1939, modifications made by Vladimir Kosma Zworykin enabled the electron microscope to be used for studying **viruses** and protein molecules. Eventually, electron microscopy was greatly improved, with microscopes able to magnify an image 2,000,000 times. One particularly interesting outcome of such research was the invention of holography and the hologram by Hungarian-born engineer Dennis Gabor in 1947. Gabor's work with this three-dimensional photography found numerous applications upon development of the laser in 1960.

There are now two distinct types of electron microscopes: the transmission variety (such as Ruska's), and the scanning variety. The Transmission Electron Microscope (TEM), developed in the 1930's, operates on the same physical principles as the light microscope but provides enhanced resolution due to the shorter wavelengths of electron beams. TEM offers resolutions to approximately 0.2 nanometers as opposed to 200 nanometers for the best light microscopes. The TEM has been used in all areas of biological and biomedical investigations because of its ability to view the finest cell structures. Scanning electron microscopes (SEM), instead of being focused by the scanner to peer through the specimen, are used to observe electrons that are scattered from the surface of the specimen as the beam contacts it. The beam is moved along the surface, scanning for any irregularities. The scanning electron microscope yields an extremely detailed three-dimensional image of a specimen but can only be used at low resolution; used in tandem, the scanning and transmission electron microscopes are powerful research tools.

Today, electron microscopes can be found in most hospital and medical research laboratories.

The advances made by Ruska, Knoll, and Hillier have contributed directly to the development of the field ion microscope (invented by Erwin Wilhelm Muller) and the scanning tunneling microscope (invented by Heinrich Rohrer and Gerd Binnig), now considered the most powerful optical tools in the world. For his work, Ruska shared the 1986 Nobel Prize for physics with Binnig and Rohrer.

See also Biotechnology; Laboratory techniques in immunology; Laboratory techniques in microbiology; Microscope and microscopy; Molecular biology and molecular genetics

ELECTRON MICROSCOPIC EXAMINATION OF MICROORGANISMS

Depending upon the **microscope** used and the preparation technique, an entire intact organism, or thin slices through the interior of the sample can be examined by electron microscopy. The electron beam can pass through very thin sections of a sample (transmission electron microscopy) or bounced off of the surface of an intact sample (scanning electron microscopy). Samples must be prepared prior to insertion into the microscope because the microscope operates in a vacuum. Biological material is comprised mainly of water and so would not be preserved, making meaningful interpretation of the resulting images impossible. For transmission electron microscopy, where very thin samples are required, the sample must also be embedded in a resin that can be sliced.

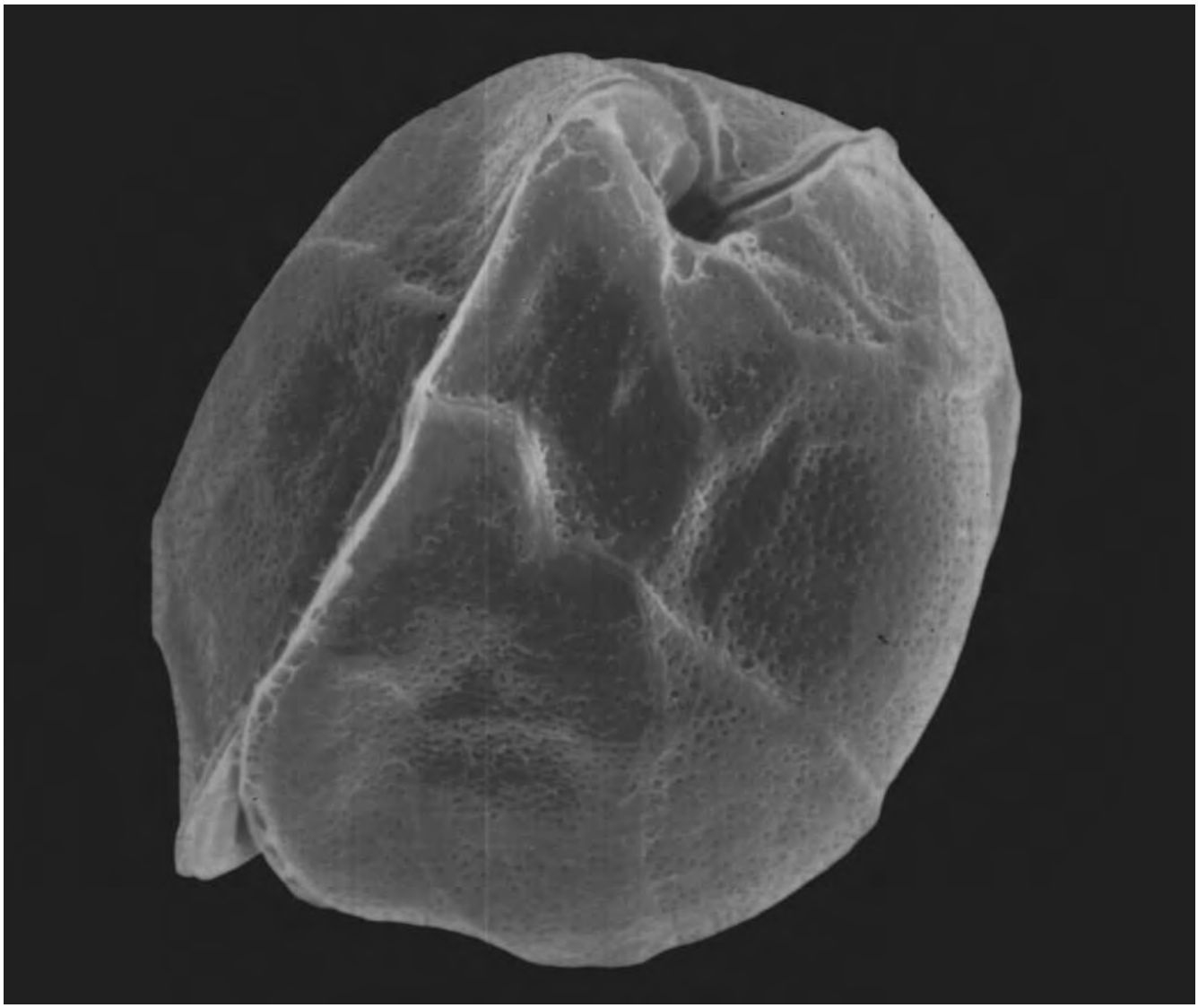
For scanning electron microscopy, a sample is coated with a metal (typically, gold) from which the incoming electrons will bounce. The deflected electrons are detected and converted to a visual image. This simple-sounding procedure requires much experience to execute properly.

Samples for transmission electron microscopy are processed differently. The sample can be treated, or fixed, with one or more chemicals to maintain the structure of the specimen. Chemicals such as glutaraldehyde or formaldehyde act to cross-link the various constituents. Osmium tetroxide and uranyl acetate can be added to increase the contrast under the electron beam. Depending on the embedding resin to be used, the water might then need to be removed from the chemically fixed specimen. In this case, the water is gradually replaced with ethanol or acetone and then the dehydrating fluid is gradually replaced with the resin, which has a consistency much like that of honey. The resin is then hardened, producing a block containing the sample. Other resins, such as Lowicryl, mix easily with water. In this case, the hydrated sample is exposed to gradually increasing concentrations of the resins, to replace the water with resin. The resin is then hardened.

Sections a few millionths of a meter in thickness are often examined by electron microscopy. The sections are sliced off from a prepared specimen in a device called a microtome, where the sample is passed by the sharp edge of a glass or diamond knife and the slice is floated off onto the surface of a volume of water positioned behind the knife-edge. The slice is gathered onto a special supporting grid. Often the section is exposed to solutions of uranyl acetate and lead citrate to further increase contrast. Then, the grid can be inserted into the microscope for examination.

Samples can also be rapidly frozen instead of being chemically fixed. This cryopreservation is so rapid that the internal water does not form structurally disruptive crystals. Frozen thin sections are then obtained using a special knife in a procedure called cryosectioning. These are inserted into the microscope using a special holder that maintains the very cold temperature.

Thin sections (both chemically fixed and frozen) and whole samples can also be exposed to antibodies in order to reveal the location of the target **antigen** within the thin section.



Scanning electron micrograph of the dinoflagellate *Gambierdiscus toxicus*.

This technique is known as immunoelectron microscopy. Care is required during the fixation and other preparation steps to ensure that the antigenic sites are not changed so that **antibody** is still capable of binding to the antigen.

Frozen samples can also be cracked open by allowing the sample to strike the sharp edge of a frozen block. The crack, along the path of least chemical resistance, can reveal internal details of the specimen. This technique is called freeze-fracture. Frozen water can be removed from the fracture (freeze-etching) to allow the structural details of the specimen to appear more prominent.

Samples such as **viruses** are often examined in the transmission **electron microscope** using a technique called negative staining. Here, sample is collected on the surface of a thin plastic support film. Then, a solution of stain is flowed over the surface. When the excess stain is carefully removed, stain will pool in the surface irregularities. Once in the microscope, electrons will not pass through the puddles of stain, producing a darker appearing region in the processed image of the specimen. Negative staining is also useful to reveal surface details of **bacteria** and appendages such as pili, flagella and spinae. A specialized form of the staining technique can also be used to visualize genetic material.

Electron microscopes exist that allow specimens to be examined in their natural, water-containing, state. Examination of living specimens has also been achieved. The so-called high-vacuum environmental microscope is finding an increasing application in the examination of microbiological samples such as **biofilms**.

See also Bacterial ultrastructure; Microscope and microscopy

ELECTRON TRANSPORT SYSTEM

The electron transport system is a coordinated series of reactions that operate in eukaryotic organisms and in prokaryotic **microorganisms**, which enables electrons to be passed from one protein to another. The purpose of the electron transport system is to pump hydrogen ions to an enzyme that utilizes the energy from the ions to manufacture the molecule known as adenine triphosphate (ATP). ATP is essentially the fuel or energy source for cellular reactions, providing the power to accomplish the many varied reactions necessary for life.

The reactions of the electron transport system can also be termed oxidative phosphorylation.

In microorganisms such as **bacteria** the machinery of the electron transport complex is housed in the single membrane of Gram-positive bacteria or in the outer membrane of Gram-negative bacteria. The electron transport process is initiated by the active, energy-requiring movement of protons (which are hydrogen ions) from the interior gel-like **cytoplasm** of the bacterium to a protein designated NADH. This protein accepts the hydrogen ion and shuttles the ion to the exterior. In doing so, the NADH is converted to NAD, with the consequent release of an electron. The released electron then begins a journey that moves it sequentially to a series of electron acceptors positioned in the membrane. Each component of the chain is able to first accept and then release an electron. Upon the electron release, the protein is ready to accept another electron. The electron transport chain can be envisioned as a coordinated and continual series of switches of its constituents from electron acceptance to electron release mode.

The energy of the electron transport system decreases as the electrons move "down" the chain. The effect is somewhat analogous to water running down a slope from a higher energy state to a lower energy state. The flow of electrons ends at the final compound in the chain, which is called ATP synthase.

The movement of electrons through the series of reactions causes the release of hydrogen to the exterior, and an increased concentration of OH^- ions (hydroxyl ions) in the interior of the bacterium.

The proteins that participate in the flow of electrons are the flavoproteins and the cytochromes. These proteins are ubiquitous to virtually all prokaryotes and **eukaryotes** that have been studied.

The ATP synthase attempts to restore the equilibrium of the hydrogen and hydronium ions by pumping a hydrogen ion back into the cell for each electron that is accepted. The energy supplied by the hydrogen ion is used to add a phosphate group to a molecule called adenine diphosphate (ADP), generating ATP.

In aerobic bacteria, which require the presence of oxygen for survival, the final electron acceptor is an atom of oxygen. If oxygen is absent, the electron transport process halts. Some bacteria have an alternate process by which energy can be generated. But, for many aerobic bacteria, the energy produced in the absence of oxygen cannot sustain bacterial survival for an extended period of time. Besides the lack of oxygen, compounds such as cyanide block the electron transport chain. Cyanide accomplishes this by binding to one of the cytochrome components of the chain. The blockage halts ATP production.

The flow of hydrogen atoms back through the membrane of bacteria and the mitochondrial membrane of eukaryotic cells acts to couple the electron transport system with the formation of ATP. Peter Mitchell, English chemist (1920–1992), proposed this linkage in 1961. He termed this the chemiosmotic theory. The verification of the mechanism proposed in the chemiosmotic theory earned Mitchell a 1978 Nobel Prize.

See also Bacterial membranes and cell wall; Bacterial ultrastructure; Biochemistry; Cell membrane transport

ELECTROPHORESIS

Protein electrophoresis is a sensitive analytical form of chromatography that allows the separation of charged molecules in a solution medium under the influence of an electric field. A wide range of molecules may be separated by electrophoresis, including, but not limited to **DNA**, **RNA,** and protein molecules.

The degree of separation and rate of molecular migration of mixtures of molecules depends upon the size and shape of the molecules, the respective molecular charges, the strength of the electric field, the type of medium used (e.g., cellulose acetate, starch gels, paper, agarose, polyacrylamide gel, etc.) and the conditions of the medium (e.g., electrolyte concentration, **pH**, ionic strength, viscosity, temperature, etc.).

Some mediums (also known as support matrices) are porous gels that can also act as a physical sieve for macromolecules.

In general, the medium is mixed with buffers needed to carry the electric charge applied to the system. The medium/buffer matrix is placed in a tray. Samples of molecules to be separated are loaded into wells at one end of the matrix. As electrical current is applied to the tray, the matrix takes on this charge and develops positively and negatively charged ends. As a result, molecules such as DNA and RNA that are negatively charged, are pulled toward the positive end of the gel.

Because molecules have differing shapes, sizes, and charges they are pulled through the matrix at different rates and this, in turn, causes a separation of the molecules. Generally, the smaller and more charged a molecule, the faster the molecule moves through the matrix.

When DNA is subjected to electrophoresis, the DNA is first broken by what are termed **restriction enzymes** that act to cut the DNA is selected places. After being subjected to restriction **enzymes**, DNA molecules appear as bands (composed of similar length DNA molecules) in the electrophoresis matrix. Because nucleic acids always carry a negative charge, separation of nucleic acids occurs strictly by molecular size.

Proteins have net charges determined by charged groups of amino acids from which they are constructed. Proteins can also be amphoteric compounds, meaning they can take on a negative or positive charge depending on the surrounding conditions. A protein in one solution might carry a positive charge

in a particular medium and thus migrate toward the negative end of the matrix. In another solution, the same protein might carry a negative charge and migrate toward the positive end of the matrix. For each protein there is an isoelectric point related to a pH characteristic for that protein where the protein molecule has no net charge. Thus, by varying pH in the matrix, additional refinements in separation are possible.

The advent of electrophoresis revolutionized the methods of protein analysis. Swedish biochemist Arne Tiselius was awarded the 1948 Nobel Prize in chemistry for his pioneering research in electrophoretic analysis. Tiselius studied the separation of serum proteins in a tube (subsequently named a Tiselius tube) that contained a solution subjected to an electric field.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis techniques pioneered in the 1960s provided a powerful means of protein fractionation (separation). Because the protein bands did not always clearly separate (i.e., there was often a great deal of overlap in the protein bands) only small numbers of molecules could be separated. The subsequent development in the 1970s of a two-dimensional electrophoresis technique allowed greater numbers of molecules to be separated.

Two-dimensional electrophoresis is actually the fusion of two separate separation procedures. The first separation (dimension) is achieved by isoelectric focusing (IEF) that separates protein polypeptide chains according to amino acid composition. IEF is based on the fact that proteins will, when subjected to a pH gradient, move to their isoelectric point. The second separation is achieved via SDS slab gel electrophoresis that separates the molecule by molecular size. Instead of broad, overlapping bands, the result of this two-step process is the formation of a two-dimensional pattern of spots, each comprised of a unique protein or protein fragment. These spots are subsequently subjected to staining and further analysis.

Some techniques involve the application of radioactive labels to the proteins. Protein fragments subsequently obtained from radioactively labels proteins may be studied my radiographic measures.

There are many variations on gel electrophoresis with wide-ranging applications. These specialized techniques include Southern, Northern, and Western blotting. Blots are named according to the molecule under study. In Southern blots, DNA is cut with restriction enzymes then probed with radioactive DNA. In Northern blotting, RNA is probed with radioactive DNA or RNA. Western blots target proteins with radioactive or enzymatically tagged antibodies.

Modern electrophoresis techniques now allow the identification of homologous DNA sequences and have become an integral part of research into **gene** structure, gene expression, and the diagnosis of heritable and autoimmune diseases. Electrophoretic analysis also allows the identification of bacterial and viral strains and is finding increasing acceptance as a powerful forensic tool.

See also Autoimmunity and autoimmune diseases; Biochemical analysis techniques; Immunoelectrophoresis

ELION, GERTRUDE BELLE (1918-1999)

American biochemist

Gertrude Belle Elion's innovative approach to drug discovery advanced the understanding of cellular **metabolism** and led to the development of medications for leukemia, gout, **herpes**, **malaria**, and the rejection of transplanted organs. Azidothymidine (AZT), the first drug approved for the treatment of **AIDS**, came out of her laboratory shortly after her retirement in 1983. One of the few women who held a top post at a major pharmaceutical company, Elion worked at Wellcome Research Laboratories for nearly five decades. Her work, with colleague George H. Hitchings, was recognized with the Nobel Prize for physiology or medicine in 1988. Her Nobel Prize was notable for several reasons: few winners have been women, few have lacked the Ph.D., and few have been industrial researchers.

Elion was born on January 23, 1918, in New York City, the first of two children, to Robert Elion and Bertha Cohen. Her father, a dentist, immigrated to the United States from Lithuania as a small boy. Her mother came to the United States from Russia at the age of fourteen. Elion, an excellent student who was accelerated two years by her teachers, graduated from high school at the height of the Great Depression. As a senior in high school, she had witnessed the painful death of her grandfather from stomach cancer and vowed to become a cancer researcher. She was able to attend college only because several New York City schools, including Hunter College, offered free tuition to students with good grades. In college, she majored in chemistry.

In 1937, Elion graduated Phi Beta Kappa from Hunter College with a B.A. at the age of nineteen. Despite her outstanding academic record, Elion's early efforts to find a job as a chemist failed. One laboratory after another told her that they had never employed a woman chemist. Her self-confidence shaken, Elion began secretarial school. That lasted only six weeks, until she landed a one-semester stint teaching **biochemistry** to nurses, and then took a position in a friend's laboratory. With the money she earned from these jobs, Elion began graduate school. To pay for her tuition, she continued to live with her parents and to work as a substitute science teacher in the New York public schools system. In 1941, she graduated summa cum laude from New York University with a M.S. degree in chemistry.

Upon her graduation, Elion again faced difficulties finding work appropriate to her experience and abilities. The only job available to her was as a quality control chemist in a food laboratory, checking the color of mayonnaise and the acidity of pickles for the Quaker Maid Company. After a year and a half, she was finally offered a job as a research chemist at Johnson & Johnson. Unfortunately, her division closed six months after she arrived. The company offered Elion a new job testing the tensile strength of sutures, but she declined.

As it did for many women of her generation, the start of World War II ushered in a new era of opportunity for Elion. As men left their jobs to fight the war, women were encouraged to join the workforce. "It was only when men weren't avail-

able that women were invited into the lab," Elion told the *Washington Post*.

For Elion, the war created an opening in the research lab of biochemist George Herbert Hitchings at Wellcome Research Laboratories in Tuckahoe, New York, a subsidiary of Burroughs Wellcome Company, a British firm. When they met, Elion was 26 years old and Hitchings was 39. Their working relationship began on June 14, 1944, and lasted for the rest of their careers. Each time Hitchings was promoted, Elion filled the spot he had just vacated, until she became head of the Department of Experimental Therapy in 1967, where she was to remain until her retirement 16 years later. Hitchings became vice president for research. During that period, they wrote many scientific papers together.

Settled in her job and encouraged by the breakthroughs occurring in the field of biochemistry, Elion took steps to earn a Ph.D., the degree that all serious scientists are expected to attain as evidence that they are capable of doing independent research. Only one school offered night classes in chemistry, the Brooklyn Polytechnic Institute (now Polytechnic University), and that is where Elion enrolled. Attending classes meant taking the train from Tuckahoe into Grand Central Station and transferring to the subway to Brooklyn. Although the hour-and-a-half commute each way was exhausting, Elion persevered for two years, until the school accused her of not being a serious student and pressed her to attend full-time. Forced to choose between school and her job, Elion had no choice but to continue working. Her relinquishment of the Ph.D. haunted her, until her lab developed its first successful drug, 6-mercaptopurine (6MP).

In the 1940s, Elion and Hitchings employed a novel approach in fighting the agents of disease. By studying the biochemistry of cancer cells, and of harmful **bacteria** and **viruses**, they hoped to understand the differences between the metabolism of those cells and normal cells. In particular, they wondered whether there were differences in how the disease-causing cells used nucleic acids, the chemicals involved in the replication of **DNA**, to stay alive and to grow. Any dissimilarity discovered might serve as a target point for a drug that could destroy the abnormal cells without harming healthy, normal cells. By disrupting one crucial link in a cell's biochemistry, the cell itself would be damaged. In this manner, cancers and harmful bacteria might be eradicated.

Elion's work focused on purines, one of two main categories of nucleic acids. Their strategy, for which Elion and Hitchings would be honored by the Nobel Prize forty years later, steered a radical middle course between chemists who randomly screened compounds to find effective drugs and scientists who engaged in basic cellular research without a thought of drug therapy. The difficulties of such an approach were immense. Very little was known about nucleic acid biosynthesis. Discovery of the double helical structure of DNA still lay ahead, and many of the instruments and methods that make **molecular biology** possible had not yet been invented. But Elion and her colleagues persisted with the tools at hand and their own ingenuity. By observing the microbiological results of various experiments, they could make knowledgeable deductions about the biochemistry involved. To the same ends, they worked with various species of lab animals and examined varying responses. Still, the lack of advanced instrumentation and computerization made for slow and tedious work. Elion told *Scientific American,* "if we were starting now, we would probably do what we did in ten years."

By 1951, as a senior research chemist, Elion discovered the first effective compound against childhood leukemia. The compound, 6-mercaptopurine (6MP; trade name Purinethol), interfered with the synthesis of leukemia cells. In clinical trials run by the Sloan-Kettering Institute (now the Memorial Sloan-Kettering Cancer Center), it increased life expectancy from a few months to a year. The compound was approved by the Food and Drug Administration (FDA) in 1953. Eventually 6MP, used in combination with other drugs and radiation treatment, made leukemia one of the most curable of cancers.

In the following two decades, the potency of 6MP prompted Elion and other scientists to look for more uses for the drug. Robert Schwartz, at Tufts Medical School in Boston, and Roy Calne, at Harvard Medical School, successfully used 6MP to suppress the immune systems in dogs with transplanted kidneys. Motivated by Schwartz and Calne's work, Elion and Hitchings began searching for other immunosuppressants. They carefully studied the drug's course of action in the body, an endeavor known as pharmacokinetics. This additional work with 6MP led to the discovery of the derivative azathioprine (Imuran), which prevents rejection of transplanted human organs and treats rheumatoid arthritis. Other experiments in Elion's lab intended to improve 6MP's effectiveness led to the discovery of allopurinol (Zyloprim) for gout, a disease in which excess uric acid builds up in the joints. Allopurinol was approved by the FDA in 1966. In the 1950s, Elion and Hitchings's lab also discovered pyrimethamine (Daraprim and Fansidar) a treatment for malaria, and trimethoprim, for urinary and respiratory tract infections. Trimethoprim is also used to treat Pneumocystis carinii **pneumonia**, the leading killer of people with AIDS.

In 1968, Elion heard that a compound called adenine arabinoside appeared to have an effect against DNA viruses. This compound was similar in structure to a chemical in her lab, 2,6-diaminopurine. Although her own lab was not equipped to screen antiviral compounds, she immediately began synthesizing new compounds to send to a Wellcome Research lab in Britain for testing. In 1969, she received notice by telegram that one of the compounds was effective against herpes simplex viruses. Further derivatives of that compound yielded acyclovir (Zovirax), an effective drug against herpes, shingles, and chickenpox. An exhibit of the success of acyclovir, presented in 1978 at the Interscience Conference on Microbial Agents and **Chemotherapy**, demonstrated to other scientists that it was possible to find drugs that exploited the differences between viral and cellular **enzymes**. Acyclovir (Zovirax), approved by the FDA in 1982, became one of Burroughs Wellcome's most profitable drugs. In 1984, at Wellcome Research Laboratories, researchers trained by Elion and Hitchings developed azidothymidine (AZT), the first drug used to treat AIDS.

Although Elion retired in 1983, she continued at Wellcome Research Laboratories as scientist emeritus and

kept an office there as a consultant. She also accepted a position as a research professor of medicine and pharmacology at Duke University. Following her retirement, Elion has served as president of the American Association for Cancer Research and as a member of the National Cancer Advisory Board, among other positions.

In 1988, Elion and Hitchings shared the Nobel Prize for physiology or medicine with Sir James Black, a British biochemist. Although Elion had been honored for her work before, beginning with the prestigious Garvan Medal of the American Chemical Society in 1968, a host of tributes followed the Nobel Prize. She received a number of honorary doctorates and was elected to the National Inventors' Hall of Fame, the National Academy of Sciences, and the National Women's Hall of Fame. Elion maintained that it was important to keep such awards in perspective. "The Nobel Prize is fine, but the drugs I've developed are rewards in themselves," she told the *New York Times Magazine*.

Elion never married. Engaged once, Elion dismissed the idea of marriage after her fiancé became ill and died. She was close to her brother's children and grandchildren, however, and on the trip to Stockholm to receive the Nobel Prize, she brought with her 11 family members. Elion once said that she never found it necessary to have women role models. "I never considered that I was a woman and then a scientist," Elion told the *Washington Post*. "My role models didn't have to be women—they could be scientists." Her other interests were photography, travel, and music, especially opera. Elion, whose name appears on 45 patents, died on February 21, 1999.

See also AIDS, recent advances in research and treatment; Antiviral drugs; Autoimmunity and autoimmune diseases; Immunosuppressant drugs; Transplantation genetics and immunology

ELISA • *see* ENZYME-LINKED IMMUNOSORBANT ASSAY (ELISA)

ENDERS, JOHN F. (1897-1985)

American virologist

John F. Enders' research on **viruses** and his advances in tissue **culture** enabled microbiologists **Albert Sabin** and **Jonas Salk** to develop vaccines against polio, a major crippler of children in the first half of the twentieth century. Enders' work also served as a catalyst in the development of vaccines against **measles**, **mumps** and chicken pox. As a result of this work, Enders was awarded the 1954 Nobel Prize in medicine or physiology.

John Franklin Enders was born February 10, 1897, in West Hartford, Connecticut. His parents were John Enders, a wealthy banker, and Harriet Whitmore Enders. Entering Yale in 1914, Enders left during his junior year to enlist in the U.S. Naval Reserve Flying Corps following America's entry into World War I in 1917. After serving as a flight instructor and rising to the rank of lieutenant, he returned to Yale, graduating in 1920. After a brief venture as a real estate agent, Enders entered Harvard in 1922 as a graduate student in English literature. His plans were sidetracked in his second year when, after seeing a roommate perform scientific experiments, he changed his major to medicine. He enrolled in Harvard Medical School, where he studied under the noted microbiologist and author Hans Zinsser. Zinsser's influence led Enders to the study of microbiology, the field in which he received his Ph.D. in 1930. His dissertation was on **anaphylaxis**, a serious allergic condition that can develop after a foreign protein enters the body. Enders became an assistant at Harvard's Department of Bacteriology in 1929, eventually rising to assistant professor in 1935, and associate professor in 1942.

Following the Japanese attack on Pearl Harbor, Enders came to the service of his country again, this time as a member of the Armed Forces **Epidemiology** Board. Serving as a consultant to the Department of War, he helped develop diagnostic tests and immunizations for a variety of diseases. Enders continued to work with the military after the war, offering his counsel to the U.S. Army's Civilian Commission on Virus and Rickettsial Disease, and the Secretary of Defense's Research and Development Board. Enders left his position at Harvard in 1946 to set up the Infectious Diseases Laboratory at Boston Children's Hospital, believing this would give him greater freedom to conduct his research. Once at the hospital, he began to concentrate on studying those viruses affecting his young patients. By 1948, he had two assistants, Frederick Robbins and **Thomas Weller**, who, like him, were graduates of Harvard Medical School. Although Enders and his colleagues did their research primarily on measles, mumps, and chicken pox, their lab was partially funded by the National Foundation for Infantile Paralysis, an organization set up to help the victims of polio and find a **vaccine** or cure for the disease. Infantile paralysis, a virus affecting the brain and nervous system was, at that time, a much-feared disease with no known prevention or cure. Although it could strike anyone, children were its primary victims during the periodic **epidemics** that swept through communities. The disease often crippled and, in severe cases, killed those afflicted.

During an experiment on chicken pox, Weller produced too many cultures of human embryonic tissue. So as not to let them go to waste, Enders suggested putting polio viruses in the cultures. To their surprise, the virus began growing in the test tubes. The publication of these results in a 1949 *Science* magazine article caused major excitement in the medical community. Previous experiments in the 1930s had indicated that the polio virus could only grow in nervous system tissues. As a result, researchers had to import monkeys in large numbers from India, infect them with polio, then kill the animals and remove the virus from their nervous system. This was extremely expensive and time-consuming, as a single monkey could provide only two or three virus samples, and it was difficult to keep the animals alive and in good health during transport to the laboratories.

The use of nervous system tissue created another problem for those working on a vaccine. Tissue from that system often stimulate allergic reactions in the brain, sometimes

fatally, when injected into another body, and there was always the danger some tissue might remain in the vaccine serum after the virus had been harvested from the culture. The discovery that the polio virus could grow outside the nervous system provided a revolutionary breakthrough in the search for a vaccine. As many as 20 specimens could be taken from a single monkey, enabling the virus to be cultivated in far larger quantities. Because no nervous system tissue had to be used, there was no danger of an allergic reaction through inadvertent transmission of the tissue. In addition, the technique of cultivating the virus and studying its effects also represented a new development in viral research. Enders and his assistants placed parts of the tissues around the inside walls of the test tubes, then closed the tubes and placed the cultures in a horizontal position within a revolving drum. Because this method made it easier to observe reaction within the culture, Enders was able to discover a means of distinguishing between the different viruses in human cells. In the case of polio, the virus killed the cell, whereas the measles virus made the cells fuse together and grow larger.

Because his breakthrough made it possible to develop a vaccine against polio, Enders, Robbins, and Weller were awarded the Nobel Prize for medicine or physiology in 1954. Interestingly enough, Enders originally opposed Salk's proposal to vaccinate against polio by injecting killed viruses into an uninfected person to produce **immunity**. He feared that this would actually weaken the immunity of the general population by interfering with the way the disease developed. In spite of their disagreements, Salk expressed gratitude to Enders by stating that he could not have developed his vaccine without the help of Enders' discoveries.

Enders' work in the field of **immunology** did not stop with his polio research. Even before he won the Nobel Prize, he was working on a vaccine against measles, again winning the acclaim of the medical world when he announced the creation of a successful vaccine against this disease in 1957. Utilizing the same techniques he had developed researching polio, he created a weakened measles virus that produced the necessary antibodies to prevent infection. Other researchers used Enders' methodology to develop vaccines against German measles and chicken pox.

In spite of his accomplishments and hard work, Enders' progress in academia was slow for many years. Still an assistant professor when he won the Nobel Prize, he did not become a full professor until two years later. This may have resulted in his dislike for university life—he once said that he preferred practical research to the "arid scholarship" of academia. Yet, by the mid-fifties, Enders began receiving his due recognition. He was given the Kyle Award from the United States Public Health Service in 1955 and, in 1962, became a university professor at Harvard, the highest honor the school could grant. Enders received the Presidential Medal of Freedom in 1963, the same year he was awarded the American Medical Association's Science Achievement Award, making him one of the few non-physicians to receive this honor.

Enders married his first wife in 1927, and in 1943, she passed away. The couple had two children. He married again in 1951. Affectionately known as "The Chief" to students and colleagues, Enders took a special interest in those he taught, keeping on the walls of his lab portraits of those who became scientists. When speaking to visitors, he was able to identify each student's philosophy and personality. Enders wrote some 190 published papers between 1929 and 1970. Towards the end of his life, he sought to apply his knowledge of immunology to the fight against **AIDS**, especially in trying to halt the progress of the disease during its incubation period in the human body. Enders died September 8, 1985, of heart failure, while at his summer home in Waterford, Connecticut.

See also Antibody and antigen; Antibody formation and kinetics; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunization; Immunochemistry; Poliomyelitis and polio

ENTAMOEBA HISTOLYTICA

Entamoeba histolytica is a eukaryotic microorganism; that is, the nuclear genetic material is enclosed within a specialized membrane. Furthermore, the microbe is a protozoan parasite. It requires a host for the completion of its life cycle, and its survival comes at the expense of the host organism. *Entamoeba histolytica* causes disease in humans. Indeed, after **malaria** and schistosomiasis, the **dysentery** caused by the amoeba is the third leading cause of death in the world. One-tenth of the world's population, some 500 million people, are infected by *Entamoeba histolytica*, with between 50,000 and 100,000 people dying of the infection each year.

The bulk of these deaths occurs in underdeveloped areas of the world, where sanitation and personal **hygiene** is lacking. In developed regions, where sanitation is established and where water treatment systems are in routine use, the dysentery caused by *Entamoeba histolytica* is almost nonexistent.

A characteristic feature of *Entamoeba histolytica* is the invasion of host tissue. Another species, *Entamoeba dispar* does not invade tissue and so does not cause disease. This nonpathogenic species does appear similar to the disease-causing species, however, which can complicate the diagnosis of the dysentery caused by *Entamoeba histolytica*.

Both **microorganisms** have been known for a long time, having been originally described in 1903. Even at that time the existence of two forms of the microorganisms were known. The two forms are called the cyst and the trophozoite. A cyst is an environmentally hardy form, designed to protect the genetic material when conditions are harsh and unfavorable for the growth of the organism. For example, cysts are found in food and water, and are the means whereby the organism is transmitted to humans. Often, the cysts are ingested in water or food that has been contaminated with the fecal material of an infected human. Within the small intestine, the cyst undergoes division of the nuclear material and then resuscitation and division of the remaining material to form eight trophozoites.

Some of the trophozoites go on to adhere to the intestinal wall and reproduce, so as to colonize the intestinal surface. The adherent trophozoites can feed on **bacteria** and cell debris that are present in the area. Some of the trophozoites are able to break down the membrane barrier of the intestinal cells

and kill these cells. The resulting abdominal pain and tenderness, with sudden and explosive bloody diarrhea, is called dysentery. Other symptoms of the dysentery include dehydration, fever, and sometimes the establishment of a bowel malfunction that can become chronic. The damage can be so extensive that a complete perforation of the intestinal wall can occur. Leakage of intestinal contents into the abdominal cavity can be a result, as can a thickening of the abdominal wall.

Other trophozoites form cysts and are shed into the external environment via the feces. These can spread the infection to another human.

Drugs are available to treat the symptomatic and asymptomatic forms of the infection.

In about 10 percent of people who are infected, some of the trophozoites can enter the circulatory system and invade other parts of the body, such as the liver, colon, and infrequently the brain. The reasons for the ability of the trophozoites to establish infections in widespread areas of the body are still not understood. The current consensus is that these trophozoites must somehow be better equipped to evade the immune responses of the host, and have more potent virulence factors capable of damaging host tissue.

Infection can occur with no obvious symptoms being shown by the infected person. However, these people will still excrete the cysts in their feces and so can spread the infection to others. In others, infection could produce no symptoms, or symptoms ranging from mild to fatal.

Although the molecular mechanisms of infection of *Entamoeba histolytica* are still unclear, it is clear that infection is a multi-stage process. In the first step the amoeba recognizes the presence of a number of surface receptors on host cells. This likely involves a reaction between the particular host receptor and a complimentary molecule on the surface of the amoeba that is known as an adhesion. Once the association between the parasite and the host intestinal cell is firm, other molecules of the parasite, which may already be present or which may be produced after adhesion, are responsible for the damage to the intestinal wall. These virulence factors include a protein that can form a hole in the intestinal wall of the host, a protein-dissolving enzyme (protease), a **glycocalyx** that covers the surface of the protozoan, and a toxin.

Comparison of pathogenic strains of *Entamoeba histolytica* with strains that look the same but which do not cause disease has revealed some differences. For example, the nonpathogenic forms have much less of two so-called glycolipids that are anchored in the microbe membrane and protrude out from the surface. Their function is not known, although they must be important to the establishment of an infection.

Completion of the sequencing of the genome of *Entamoeba histolytica*, expected by 2005, should help identify the function of the suspected virulence factors, and other, yet unknown, virulence factors. Currently, little is known of the genetic organization and regulation of expression of the genetic material in the protozoan. For example, the reasons for the variation in the infection and the symptoms are unclear.

See also Amebic dysentery; Parasites

ENTEROBACTERIACEAE

Enterobacteria are **bacteria** from the family Enterobacteriaceae, which are primarily known for their ability to cause intestinal upset. Enterobacteria are responsible for a variety of human illnesses, including urinary tract infections, wound infections, **gastroenteritis**, **meningitis**, septicemia, and **pneumonia**. Some are true intestinal pathogens; whereas others are merely opportunistic pests which attack weakened victims.

Most enterobacteria reside normally in the large intestine, but others are introduced in contaminated or improperly prepared foods or beverages. Several enterobacterial diseases are spread by fecal-oral transmission and are associated with poor hygienic conditions. Countries with poor water decontamination have more illness and death from enterobacterial infection. Harmless bacteria, though, can cause diarrhea in tourists who are not used to a geographically specific bacterial strain. Enterobacterial gastroenteritis can cause extensive fluid loss through vomiting and diarrhea, leading to dehydration.

Enterobacteria are a family of rod-shaped, aerobic, facultatively anaerobic bacteria. This means that while these bacteria can survive in the presence of oxygen, they prefer to live in an anaerobic (oxygen-free) environment. The Enterobacteriaceae family is subdivided into eight tribes including: Escherichieae, Edwardsielleae, Salmonelleae, Citrobactereae, Klebsielleae, Proteeae, Yersineae, and Erwineae. These tribes are further divided into genera, each with a number of species.

Enterobacteria can cause disease by attacking their host in a number of ways. The most important factors are motility, colonization factors, endotoxin, and **enterotoxin**. Those enterobacteria that are motile have several flagella all around their perimeter (peritrichous). This allows them to move swiftly through their host fluid. Enterobacterial colonization factors are filamentous appendages, called fimbriae, which are shorter than flagella and bind tightly to the tissue under attack, thus keeping hold of its host. Endotoxins are the cell wall components, which trigger high fevers in infected individuals. Enterotoxins are bacterial toxins which act in the small intestines and lead to extreme water loss in vomiting and diarrhea.

A number of tests exist for rapid identification of enterobacteria. Most will ferment glucose to acid, reduce nitrate to nitrite, and test negative for cytochrome oxidase. These biochemical tests are used to pin-point specific intestinal pathogens. ***Escherichia coli (E. coli), Shigella*** *species,* ***Salmonella****,* and several *Yersinia* strains are some of these intestinal pathogens.

E. coli is indigenous to the gastrointestinal tract and generally benign. However, it is associated with most hospital-acquired infections as well as nursery and travelers diarrhea. *E. coli* pathogenicity is closely related to the presence or absence of fimbriae on individual strains. Although most *E. coli* infections are not treated with **antibiotics**, severe urinary tract infections usually are.

The *Shigella* genus of the Escherichieae tribe can produce serious disease when its toxins act in the small intestine. *Shigella* infections can be entirely asymptomatic, or lead to severe **dysentery**. *Shigella* bacteria cause about 15% of pedi-

atric diarrheal cases in the United States. However, they are a leading cause of infant mortality in developing countries. Only a few organisms are need to cause this fecal-orally transmitted infection. Prevention of the disease is achieved by proper sewage disposal and water **chlorination**, as well as personal **hygiene** such as handwashing. Antibiotics are only used in more severe cases.

Salmonella infections are classified as nontyphoidal or typhoidal. Nontyphoidal infections can cause gastroenteritis, and are usually due to contaminated food or water and can be transmitted by animals or humans. These infections cause one of the largest communicable bacterial diseases in the United States. They are found in contaminated animal products such as beef, pork, poultry, and raw chicken eggs. As a result, any food product that uses raw eggs, such as mayonnaise, homemade ice cream, or Caesar salad, could carry these bacteria. The best prevention when serving these dishes is to adhere strictly to refrigeration guidelines.

Typhoid *Salmonella* infections are also found in contaminated food and water. Typhoid Mary was a cook in New York from 1868 to 1914. She was typhoid carrier who contaminated much of the food she handled and was responsible for hundreds of typhoid cases. **Typhoid fever** is characterized by septicemia (blood poisoning), accompanied by a very high fever and intestinal lesions. Typhoid fever is treated with the drugs Ampicillin and Chloramphenicol.

Certain *Yersinia* bacteria cause one of the most notorious and fatal infections known to man. *Yersinia pestis* is the agent of **bubonic plague** and is highly fatal without treatment. The bubonic plague is carried by a rat flea and is thought to have killed at least 100 million people in the sixth century as well as 25% of the fourteenth century European population. This plague was also known as the "black death," because it caused darkened hemorrhagic skin patches. The last widespread epidemic of *Y. pestis* began in Hong Kong in 1892 and spread to India and eventually San Francisco in 1900. The bacteria can reside in squirrels, prairie dogs, mice, and other rodents, and are mainly found (in the U.S.) in the Southwest. Since 1960, fewer than 400 cases have resulted in only a few deaths, due to rapid antibiotic treatment.

Two less severe *Yersinia* strains are *Y. pseudotuberculosis* and *Y. enterocolotica*. *Y. pseudotuberculosis* is transmitted to humans by wild or domestic animals and causes a non-fatal disease which resembles appendicitis. *Y. enterocolotica* can be transmitted from animals or humans via a fecal-oral route and causes severe diarrhea.

See also Colony and colony formation; Enterobacterial infections; Infection and resistance; Microbial flora of the stomach and gastrointestinal tract

ENTEROBACTERIAL INFECTIONS

Enterobacterial infections are caused by a group of **bacteria** that dwell in the intestinal tract of humans and other warm-blooded animals. The bacteria are all Gram-negative and rod-shaped. As a group they are termed **Enterobacteriaceae**. A prominent member of this group is ***Escherichia coli***. Other members are the various species in the genera ***Salmonella, Shigella***, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, and *Yersinia*.

The various enterobacteria cause intestinal maladies. As well, if they infect regions of the body other than their normal intestinal habitat, infections can arise. Often, the **bacterial infection** arises during the course of a hospital stay. Such infections are described as being nosocomial, or hospital acquired, infections. For example, both *Klebsiella* and *Proteus* are capable of establishing infections in the lung, ear, sinuses, and the urinary tract if they gain entry to these niches. As another example, both *Enterobacter* and *Serratia* can cause an infection of the blood, particularly in people whose immune systems are compromised as a result of therapy or other illness.

A common aspect of enterobacterial infections is the presence of diarrhea. Indeed, the diarrhea caused by enterobacteria is a common problem even in countries like the United States, which has an excellent medical infrastructure. In the United States is has been estimated that each person in the country experiences 1.5 episodes of diarrhea each year. While for most of those afflicted the diarrhea is a temporary inconvenience, those who are young, old, or whose immune systems are malfunctioning can be killed by the infection. Moreover, in other countries where the medical facilities are less advanced, enterobacterial infections remain a serious health problem.

Even in the intestinal tract, where they normally reside, enterobacteria can cause problems. Typically, intestinal maladies arise from types of the enterobacteria that are not part of the normal flora. An example is ***E. coli* O157:H7**. While this bacterial strain is a normal resident in the intestinal tract of cattle, its presence in the human intestinal tract is abnormal and problematic.

The O157:H7 strain establishes an infection by invading host tissue. Other bacteria, including other strains of *Escherichia coli*, do not invade host cells. Rather, they adhere to the intestinal surface of the cells and can exert their destructive effect by means of toxins they elaborate. Both types of infections can produce diarrhea. Bloody diarrhea (which is also known as **dysentery**) can result when host cells are damaged. Some types of *Escherichia coli*, *Salmonella*, and *Shigella* produce dysentery.

Escherichia coli O157:H7 can also become disseminated in the blood and cause destruction of red blood cells and impaired or complete loss of function of the kidneys. This debilitating and even life-threatening infection is known as hemolytic-uremic syndrome.

Another intestinal upset that occurs in prematurely born infants is called necrotizing enterocolitis. Likely the result of a bacterial (or perhaps a viral) infection, the cells lining the bowel is killed. In any person such an infection is serious. But in a prematurely borne infant, whose **immune system** is not able to deal with an infection, necrotizing enterocolitis can be lethal. The enterobacteria that have been associated with the disease are *Salmonella*, *Escherichia coli*, *Klebsiella*, and *Enterobacter*.

The diagnosis of enterobacterial infections can be complicated by the fact that **viruses**, **protozoa**, and other kinds of bacteria can also cause similar symptoms. The location of some of the symptoms can help determine the nature of the infection. For example, if nausea and vomiting is involved, then the enterobacterial infection could well be centered in the small intestine. If a fever is present, then dysentery is more likely.

The treatment for many enterobacterial infections is the administration of the suitable antibiotic or combination of **antibiotics** that the isolated organism is determined to be susceptible to. As well, and every bit as important, is the administration of fluids to prevent dehydration because of the copious loss of fluids during diarrhea. The dehydration can be extremely debilitating to infants and the elderly.

See also E. coli O157:H7 infection; Invasiveness and intracellular infections

ENTEROTOXIN AND EXOTOXIN

Enterotoxin and exotoxin are two classes of toxin that are produced by **bacteria**.

An exotoxin is a toxin that is produced by a bacterium and then released from the cell into the surrounding environment. The damage caused by an exotoxin can only occur upon release. As a general rule, enterotoxins tend to be produced by Gram-positive bacteria rather than by Gram-negative bacteria. There are exceptions, such as the potent enterotoxin produced by *Vibrio cholerae*. In contrast to Gram-positive bacteria, many Gram-negative species posses a molecule called lipopolysaccharide. A portion of the lipopolysaccharide, called the lipid A, is a cell-associated toxin, or an endotoxin.

An enterotoxin is a type of exotoxin that acts on the intestinal wall. Another type of exotoxin is a neurotoxin. This type of toxin disrupts nerve cells.

Many kinds of bacterial enterotoxins and exotoxins exist. For example, an exotoxin produced by *Staphylococcus aureus* is the cause of **toxic shock syndrome**, which can produce symptoms ranging from nausea, fever and sore throat, to collapse of the central nervous and circulatory systems. As another example, *Staphylococcus aureus* also produces enterotoxin B, which is associated with food-borne illness. Growth of the bacteria in improperly handled foods leads to the excretion of the enterotoxin. Ingestion of the toxin-contaminated food produces fever, chills, headache, chest pain and a persistent cough. This type of illness is known as a food intoxication, to distinguish it from bacterial food-borne illness that results from growth of the bacteria following ingestion of the food (food poisoning).

Enterotoxins have three different basis of activity. One type of enterotoxin, which is exemplified by **diphtheria** toxin, causes the destruction of the host cell to which it binds. Typically, the binding of the toxin causes the formation of a hole, or pore, in the host cell membrane. Another example of a pore-forming exotoxin is the aerolysin produced by *Aeromonas hydrophila*.

A second type of enterotoxin is known as a superantigen toxin. Superantigen exotoxins work by overstimulating the immune response, particularly with respect to the T-cells. Examples of superantigen exotoxins include that from *Staphylococcus aureus* and from the "flesh-eating" bacterium *Streptococcus pyogenes*.

A third type of enterotoxin is known as an A-B toxin. An A-B toxin consists of two or more toxin subunits that work together as a team to exert their destructive effect. Typically, the A subunit binds to the host cell wall and forms a channel through the membrane. The channel allows the B subunit to get into the cell. An example of an A-B toxin is the enterotoxin that is produced by *Vibrio cholerae*.

The cholera toxin disrupts the ionic balance of the host's intestinal cell membranes. As a result, the cells of the small intestine exude a large amount of water into the intestine. Dehydration results, which can be lethal if not treated.

In contrast to the destructive effect of some exotoxins, the A-B exotoxin (an enterotoxin of *Vibrio cholerae* does not damage the structure of the affected host cells. Therefore, in the case of the cholera toxin, treatment can led to a full resumption of host cell activity.

See also Anthrax, terrorist use of as a biological weapon; Bacteria and bacterial infection

ENTEROVIRUS INFECTIONS

Enteroviruses are a group of **viruses** that contain **ribonucleic acid** as their genetic material. They are members of the picornavirus family. The various types of enteroviruses that infect humans are referred to as serotypes, in recognition of their different antigenic patterns. The different immune response is important, as infection with one type of enterovirus does not necessarily confer protection to infection by a different type of enterovirus. There are 64 different enterovirus serotypes. The serotypes include polio viruses, coxsackie A and B viruses, echoviruses and a large number of what are referred to as non-polio enteroviruses.

The genetic material is enclosed in a shell that has 20 equilateral triangles (an icosahedral virus). The shell is made up of four proteins.

Despite the diversity in the antigenic types of enterovirus, the majority of enterovirus cases in the United States is due to echoviruses and Coxsackie B viruses. The infections that are caused by these viruses are varied. The paralytic debilitation of polio is one infection. The importance of polio on a global scale is diminished now, because of the advent and worldwide use of polio vaccines. Far more common are the **cold**-like or flu-like symptoms caused by various enteroviruses. Indeed, the non-polio enteroviruses rival the cause of the "common cold," the rhinovirus, as the most common infectious agent in humans. In the United States, estimates from the **Centers for Disease Control** are that at least ten to fifteen million people in the United States develop an enterovirus infection each year.

Enterovirus infection is spread easily, as the virus is found in saliva, sputum or nasal secretions, and also in the feces of those who are infected. Humans are the only known reservoir of enteroviruses. Following spread to water via feces, enteroviruses can persist in the environment. Thus, surface and ground water can be a source of enterovirus.

Spread of an enterovirus occurs by direct contact with the fluids from an infected person, by use of utensils that have been handled by an infected person, or by the ingestion of contaminated food or water. For example, coughing into someone's face is an easy way to spread enterovirus, just as the cold-causing rhinoviruses are spread from person to person. Fecal contact is most common in day care facilities or in households where there is a newborn, where diapers are changed and soiled babies cleaned up.

The spread of enterovirus infections is made even easier because some of those who are infected do not display any symptoms of illness. Yet such people are still able to transfer the infectious virus to someone else.

The common respiratory infection can strike anyone, from infants to the elderly. The young are infected more frequently, however, and may indeed be the most important transmitters of the virus. Common symptoms of infection include a runny nose, fever with chills, muscle aches and sometimes a rash. In addition, but more rarely, an infection of the heart (endocarditis), meninges (**meningitis**) or the brain (encephalitis) can develop. In newborns, enterovirus infection may be related to the development of juvenile-onset diabetes, and, in rare instances, can lead to an overwhelming infection of the body that proves to be lethal.

Although enterovirus-induced meningitis is relatively rare, it afflicts between 30,000 and 50,000 people each year in the United States alone.

Evidence is accumulating that suggests that enterovirus infections may not only be short in duration (also referred as acute) but may also become chronic. Diseases such as chronic heart disease and chronic fatigue syndrome may well have an enterovirus origin. Moreover, juvenile diabetes may involve an autoimmune response.

The climate affects the prevalence of the infections. In tropical climates, where warm temperatures are experienced throughout the year, enterovirus infections occur with similar frequency year-round. But, in more temperate climates, where a shift in seasons is pronounced, enterovirus infections peak in the late summer and fall.

Another factor in the spread of enterovirus infections is the socio-economic conditions. Poor sanitation that is often coincident with lower economic standing is often associated with the spread of enterovirus infections.

Following inhalation or ingestion of enterovirus, viral replication is thought to occur mainly in lymphoid tissues of the respiratory and gastrointestinal tract that are in the immediate vicinity of the virus. Examples of tissues include the tonsils and the cells lining the respiratory and intestinal tracts. The virus may continue to replicate in these tissues, or can spread to secondary sites including the spinal cord and brain, heart or the skin.

As with other viruses, enteroviruses recognize a receptor molecule on the surface of host cells and attach to the receptor via a surface molecule on the virus particle. Several viral molecules have been shown to function in this way. The virus then enters the host cell and the genetic material is released into the **cytoplasm** (the interior gel-like region) of the host cell. The various steps in viral replication cause, initially, the host cell **nucleus** to shrink, followed by shrinkage of the entire. Other changes cause the host cell to lose its ability to function and finally to explode, which releases newly made virus.

Currently, no **vaccine** exists for the maladies other than polio. One key course of action to minimize the chances of infection is the observance of proper **hygiene**. Handwashing is a key factor in reducing the spread of many microbial infections, including those caused by enteroviruses. Spread of enteroviruses is also minimized by covering the mouth when coughing and the nose when sneezing.

See also Cold, common; Viruses and responses to viral infection

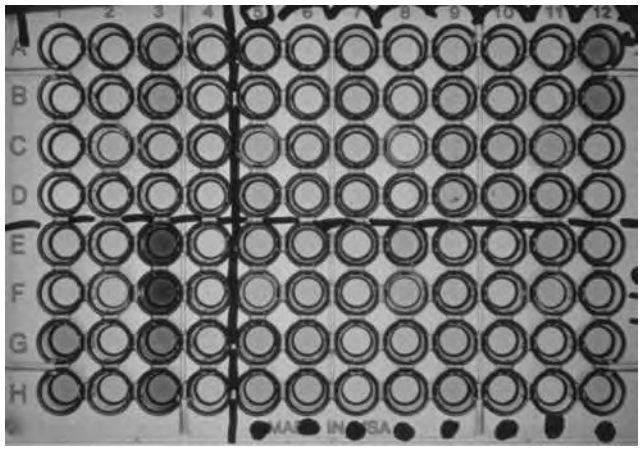
ENZYME-LINKED IMMUNOSORBANT ASSAY (ELISA)

The enzyme-linked immunosorbant assay, which is commonly abbreviated to ELISA, is a technique that promotes the binding of the target antigen or **antibody** to a substrate, followed by the binding of an enzyme-linked molecule to the bound antigen or antibody. The presence of the antigen or antibody is revealed by color development in a reaction that is catalyzed by the enzyme which is bound to the antigen or antibody.

Typically, an ELISA is performed using a plastic plate which contains an 8 x 12 arrangement of 96 wells. Each well permits a sample to be tested against a whole battery of antigens.

There are several different variations on the ELISA theme. In the so-called direct ELISA, the antigen that is fixed to the surface of the test surface is the target for the binding of a complimentary antibody to which has been linked an enzyme such as horseradish peroxidase. When the substrate of the enzyme is added, the conversion of the substrate to a colored product produces a darkening in whatever well an antigen-antibody reaction occurred.

Another ELISA variation is known as the indirect technique. In this technique a specific antibody recognizes the antigen that is bound to the bottom of the wells on the plastic plate. Binding between the antigen and the antibody occurs. The bound antibody can then be recognized by a second antibody, to which is fixed the enzyme that produces the color change. For example, in this scheme the first, or primary, antibody could be a rabbit antibody to the particular antigen. The so-called secondary antibody could be a goat-antirabbit antibody. That is, the primary antibody has acted as an antigen to produce an antibody in a second animal. Once again, the darkening of a well indicates the formation of a complex between the antigen and the antibodies.



ELISA assay 96 well test plate.

The third variation of the ELISA is known as the capture or sandwich ELISA. As the names imply, the antigen is sandwiched between the primary and secondary antibodies. In this technique, the primary antibody is bound to the bottom of the wells, rather than the antigen. Then, the antigen is added. Where the bound antibody recognizes the antigen, binding occurs. A so-called blocking solution is added, which occupies the vacant antibody sites. Then, an enzyme-labeled secondary antibody is added. The secondary antibody also recognizes the antigen, but the antigenic recognition site is different than that recognized by the primary antibody. The result is that the antigen is sandwiched in between two bound antibodies. Again, a color reaction reveals the complex.

The ELISA procedure has many applications. The procedure can provide qualitative ("yes or no") and quantitative ("how much") information on a myriad of prokaryotic and eukaryotic antibodies. Serum can be screened against a battery of antigens in order to rapidly assess the range of antibodies that might be present. For example, ELISA has proven very useful in the scrutiny of serum for the presence of antibodies to the **Human immunodeficiency virus**.

See also Laboratory techniques in immunology

A technician adds blood samples to a multi-welled sample tray during an Enzyme-linked ImmunoSorbent Assay (ELISA) test for viral diseases such as AIDS and Hepatitis B and C. Blood serum of the patient is added to burst T cells of blood that have been infected with disease. A color change occurrs if viral antibodies are present.

Enzyme induction and repression

Microorganisms have many **enzymes** that function in the myriad of activities that produce a growing and dividing cell. From a health standpoint, some enzymes are vital for the establishment of an infection by the microbes. Some enzymes are active all the time. These are known as constitutive enzymes. However, other enzymes are active only periodically, when their product is required. Such enzymes are known as inducible enzymes.

The ability of microorganisms such as **bacteria** to control the activity of inducible enzymes is vital for their survival. The constant activity of such enzymes could result in the overproduction of a compound, which would be an energy drain on the microorganism. At the same time, inducible enzymes must be capable of a rapid response to whatever condition they are geared to respond.

The twin goals of control of activity and speed of response are achieved by the processes of induction and repression.

Induction and repression are related in that they both focus on the binding of a molecule known as **RNA** polymerase to **DNA**. Specifically, the RNA polymerase binds to a region that is immediately "upstream" from the region of DNA that codes for a protein. The binding region is termed the operator. The operator acts to position the polymerase correctly, so that the molecule can then begin to move along the DNA, interpreting the genetic information as it moves along.

The three-dimensional shape of the operator region influences the binding of the RNA polymerase. The configuration of the operator can be altered by the presence of molecules called effectors. An effector can alter the shape of the polymerase-binding region so that the polymerase is more eas-

ily and efficiently able to bind. This effect is called induction. Conversely, effectors can associate with the operator and alter the configuration so that the binding of the polymerase occurs less efficiently or not at all. This effect is known as repression.

Enzyme induction is a process where an enzyme is manufactured in response to the presence of a specific molecule. This molecule is termed an inducer. Typically, an inducer molecule is a compound that the enzyme acts upon. In the induction process, the inducer molecule combines with another molecule, which is called the repressor. The binding of the inducer to the repressor blocks the function of the repressor, which is to bind to a specific region called an operator. The operator is the site to which another molecule, known as **ribonucleic acid** (RNA) polymerase, binds and begins the **transcription** of the **gene** to produce the so-called messenger RNA that acts as a template for the subsequent production of protein. Thus, the binding of the inducer to the repressor keeps the repressor from preventing transcription, and so the gene coding for the inducible enzyme is transcribed. Repression of transcription is essentially the default behavior, which is overridden once the inducing molecule is present.

In bacteria, the lactose (lac) **operon** is a very well characterized system that operates on the basis of induction.

Enzyme repression is when the repressor molecules prevent the manufacture of an enzyme. Repression typically operates by feedback inhibition. For example, if the end product of a series of enzyme-catalyzed reactions is a particular amino acid, that amino acid acts as the repressor molecule to further production. Often the repressor will combine with another molecule and the duo is able to block the operation of the operator. This blockage can occur when the repressor duo outcompetes with the polymerase for the binding site on the operator. Alternately, the repressor duo can bind directly to the polymerase and, by stimulating a change in the shape of the polymerase, prevent the subsequent binding to the operator region. Either way, the result is the blockage of the transcription of the particular gene.

The gene that is blocked in enzyme repression tends to be the first enzyme in the pathway leading to the manufacture of the repressor. Thus, repression acts to inhibit the production of all the enzymes involved in the metabolic pathway. This saves the bacterium energy. Otherwise, enzymes would be made—at a high metabolic cost—for which there would be no role in cellular processes.

Induction and repression mechanisms tend to cycle back and forth in response to the level of effector, and in response to nutrient concentration, **pH**, or other conditions for which the particular effector is sensitive.

See also Metabolism; Microbial genetics

ENZYMES

Enzymes are molecules that act as critical catalysts in biological systems. Catalysts are substances that increase the rate of chemical reactions without being consumed in the reaction. Without enzymes, many reactions would require higher levels of energy and higher temperatures than exist in biological systems. Enzymes are proteins that possess specific binding sites for other molecules (substrates). A series of weak binding interactions allow enzymes to accelerate reaction rates. Enzyme kinetics is the study of enzymatic reactions and mechanisms. Enzyme inhibitor studies have allowed researchers to develop therapies for the treatment of diseases, including **AIDS**.

French chemist **Louis Pasteur** (1822–1895) was an early investigator of enzyme action. Pasteur hypothesized that the conversion of sugar into alcohol by **yeast** was catalyzed by "ferments," which he thought could not be separated from living cells. In 1897, German biochemist Eduard Buchner (1860–1917) isolated the enzymes that catalyze the **fermentation** of alcohol from living yeast cells. In 1909, English physician Sir Archibald Garrod (1857–1936) first characterized enzymes genetically through the one gene-one enzyme hypothesis. Garrod studied the human disease alkaptonuria, a hereditary disease characterized by the darkening of excreted urine after exposure to air. He hypothesized that alkaptonurics lack an enzyme that breaks down alkaptans to normal excretion products, that alkaptonurics inherit this inability to produce a specific enzyme, and that they inherit a mutant form of a **gene** from each of their parents and have two mutant forms of the same gene. Thus, he hypothesized, some genes contain information to specify particular enzymes.

The early twentieth century saw dramatic advancement in enzyme studies. German chemist Emil Fischer (1852–1919) recognized the importance of substrate shape for binding by enzymes. German-American biochemist Leonor Michaelis (1875–1949) and Canadian biologist Maud Menten (1879–1960) introduced a mathematical approach for quantifying enzyme-catalyzed reactions. American chemists James Sumner (1887–1955) and John Northrop (1891–1987) were among the first to produce highly ordered enzyme crystals and firmly establish the proteinaceous nature of these biological catalysts. In 1937, German-born British biochemist **Hans Krebs** (1900–1981) postulated how a series of enzymatic reactions were coordinated in the citric acid cycle for the production of ATP from glucose metabolites. Today, enzymology is a central part of biochemical study, and the fields of industrial microbiology and genetics employ enzymes in numerous ways, from food production to gene **cloning**, to advanced therapeutic techniques.

Enzymes are proteins that encompass a large range of molecular size and mass. They may be composed of more than one polypeptide chain. Each polypeptide chain is called a subunit and may have a separate catalytic function. Some enzymes require non-protein groups for enzymatic activity. These components include metal ions and organic molecules called coenzymes. Coenzymes that are tightly or covalently attached to enzymes are termed prosthetic groups. Prosthetic groups contain critical chemical groups which allow the overall catalytic event to occur.

Enzymes bind their substrates at special folds and clefts in their structures called active sites. Because active sites have chemical groups precisely located and orientated for binding the substrate, they generally display a high degree of substrate

specificity. The active site of an enzyme consists of two key regions, the catalytic site, which interacts with the substrate during the reaction, and the binding site, the chemical groups of the enzyme that bind the substrate, allowing the interactions at the catalytic site to occur. The crevice of the active site creates a microenvironment whose properties are critical for catalysis. Environmental factors influencing enzyme activity include **pH**, polarity and hydrophobicity of amino acids in the active site, and a precise arrangement of the chemical groups of the enzyme and its substrate.

Enzymes have high catalytic power, high substrate specificity, and are generally most active in aqueous solvents at mild temperature and physiological pH. Most enzymes catalyze the transfer of electrons, atoms, or groups of atoms. There are thousands of known enzymes, but most can be categorized according to their biological activities into six major classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.

Enzymes generally have an optimum pH range in which they are most active. The pH of the environment will affect the ionization state of catalytic groups at the active site and the ionization of the substrate. Electrostatic interactions are therefore controlled by pH. The pH of a reaction may also control the conformation of the enzyme by influencing amino acids critical for the three-dimensional shape of the macromolecule.

Inhibitors can diminish the activity of an enzyme by altering the binding of substrates. Inhibitors may resemble the structure of the substrate, thereby binding the enzyme and competing for the correct substrate. Inhibitors may be large organic molecules, small molecules, or ions. They can be used for chemotherapeutic treatment of diseases.

Regulatory enzymes are characterized by increased or decreased activity in response to chemical signals. Metabolic pathways are regulated by controlling the activity of one or more enzymatic steps along that path. Regulatory control allows cells to meet changing demands for energy and metabolites.

See also Biochemical analysis techniques; Biotechnology; Bioremediation; Cloning, application of cloning to biological problems; Enzyme induction and repression; Enzyme-linked immunosorbant assay (ELISA); Food preservation; Food safety; Immunologic therapies; Immunological analysis techniques

EPIDEMICS AND PANDEMICS

Epidemics are outbreaks of disease of bacterial or viral origin that involve many people in a localized area at the same time. An example of an epidemic is the hemorrhagic fever outbreak caused by the **Ebola virus** in Zaire in 1976. When Ebola fever occurs, it tends to be confined to a localized area, and can involve many people. If an outbreak is worldwide in scope, it is referred to as a pandemic. The periodic outbreaks of **influenza** can be pandemic.

Some maladies can be both epidemic and pandemic. This can be a function of time. An example is Acquired **Immunodeficiency** Syndrome (**AIDS**). Initially, the acknowledged viral agent of AIDS, the **Human Immunodeficiency Virus** (**HIV**), was prevalent in a few geographic regions, such as Haiti, and among certain groups, such as homosexual men in the United States. In these regions and populations, the infection was epidemic in scope. Since these early days, AIDS has expanded to become a worldwide disease that cuts across all racial, cultural, economic and geographic categories. AIDS is now a pandemic.

Influenza can also be epidemic or pandemic. In this case, the antigenic composition of the viral agent of the disease determines whether the virus becomes global in its distribution or not. Antigenic variants of the virus that are quite different from varieties that have preceded it, and so require an adaptive response by the **immune system** before the infection can be successfully coped with, tend to become pandemic.

Pandemics of influenza can be devastating. The huge number of people who become ill can tax the capability of a regions' or countries' health infrastructure. The preparation to attempt to thwart an influenza pandemic is immense. For example, the preparation and distribution of the required **vaccine**, and the subsequent inoculation of those who might be at risk, is a huge undertaking. In human terms, influenza pandemics exact a huge toll in loss of life. Even thought the death rate from influenza is typically less than one percent of those who are infected, a pandemic involving hundreds of millions of people will result in a great many deaths.

Epidemics and pandemics have been a part of human history for millennia. An example of this long-standing presence is cholera. Cholera is an infection that is caused by a bacterium called *Vibrio cholerae*. The bacterium is present in the feces, and can be spread directly to drinking water, and to food via handling of the food in an unhygienic manner. The resulting watery diarrhea and dehydration, which can lead to collapse of body functions and death if treatment is not prompt, has devastated populations all over the world since the beginning of recorded history. The first reports that can be identified as cholera date back to 1563 in India. This and other epidemics in that part of the world lead to the spread of the infection. By 1817 cholera had become pandemic. The latest cholera pandemic began in 1961 in Indonesia. The outbreak spread through Europe, Asia, Africa, and finally reached South America in the early 1990s. In Latin America, cholera still causes 400,000 cases of illness and over 4000 deaths each year.

Influenza is another example of am illness that has been present since antiquity. Indeed, the philosopher Hippocrates first described an influenza outbreak in 412 B.C. There were three major outbreaks of influenza in the sixteenth century (the one occurring in 1580 being a pandemic), and at least three pandemics in the eighteenth century. In the twentieth century there were pandemics in 1918, 1957, and 1968. These were caused by different antigenic types of the influenza virus. The 1918 pandemic is thought to have killed some 30 million people, more than were killed in World War I.

A common theme of epidemics and pandemics throughout history has been the association of outbreaks and sanitary

A painting depicting the effect of an epidemic (in this case, the plague in Florence, Italy).

conditions. Inadequate sanitation has and continues to be the breeding ground for the **bacteria** and **viruses** that can sweep through populations. The gathering of people in the burgeoning cities of seventeenth and eighteenth century Europe lead to a series of epidemics. These included **typhus**, **typhoid fever**, plague, **smallpox**, **dysentery**, and cholera. Outbreaks are less of a problem in modern day cities, due to better sanitation conditions and standards of housing. However, in underdeveloped areas of the world, or even in the developed world where sanitation and housing conditions are deficient, such diseases are still present.

Epidemics and pandemics can be so devastating that they can alter the course of history. An example is the Black Plague that spread through Europe and Britain in the seventeenth century. An estimated one-third of the population of Europe was killed, and cities such as London became nearly deserted, as those who could afford to do so fled the city. In the Crimean War (1853–1856), more than 50,000 soldiers died of typhus, while only 2,000 soldiers were actually killed in battle. As a final example, the spread of the plague to the New World by contaminated blankets aboard French sailboats that docked at Halifax, Nova Scotia, in 1746, lead to the decimation of the aboriginal inhabitants of North America.

Within the past several decades, there has been an increasing recognition that disease that were previously assumed to be of genetic or other, nonbacterial or nonviral origin are in fact caused by **microorganisms** has lead to the recognition that there may be an epidemic or pandemic or maladies such as stomach ulcers and heart disease. These diseases differ from other bacterial and viral epidemics and pandemics, because they do not appear and fade over a relatively short time. Rather, the stomach ulcers caused by the bacterium *Helicobacter pylori* and the heart disease caused by the reaction of the immune system to infection by the bacterium *Chlamydia* are so-called chronic infections. These infections are present for a long time, essentially causing a non-stop pandemic of the particular malady.

See also Bacteria and bacterial infections; Bubonic plague; Flu, Great flu epidemic of 1918

Court held outside during an epidemic, to lessen the chance of spread of illness.

EPIDEMICS, BACTERIAL

An epidemic is the occurrence of an illness among a large number of people in the same geographical area at the same time. Bacterial **epidemics** have probably been part of the lives of humans since the species evolved millions of years ago. Certainly by the time humans were present, **bacteria** were well established.

On example of a bacterial epidemic is the plague. Plague is caused by the bacterium *Yersinia pestis*. The bacterium lives in a type of rodent flea and is transmitted to people typically via the bite of the flea. People who come into contact with an infected animal or a flea-infested animals such as a rat can also contract the disease.

Plague has been a scourge on human populations for centuries. In the Middle Ages, the so-called Black Plague (**Bubonic plague**) killed millions of people in Europe. The crowded living conditions and poor sanitation that were typical of the disadvantaged populations of the large European cities of that time were breeding grounds for the spread of plague.

While often thought of as an epidemic of the past, plague remains today. Indeed, in the United States the last epidemic of plague occurred as recently as 1924–1925 in Los Angeles. The widespread use of **antibiotics** has greatly reduced the incidence of plague. Nonetheless, the potential for an epidemic remains.

As for plague, the use of antibiotics has reduced bacterial epidemics. However, this reduction generally tends to be a feature of developed regions of the world and regions that have ready access to health care. In other less advantaged areas of the globe, bacterial epidemics that have been largely conquered in North America and Europe, for example, still claim many lives.

An example is bacterial **meningitis**. The bacterial form of meningitis (an infection of the fluid in the spinal cord and surrounding the brain) is caused by *Haemophilus influenzae* type b, *Neisseria meningitides*, and *Streptococcus pneumoniae*. Antibiotics that are routinely given to children as part of

the series of inoculations to establish **immunity** to the infection readily kill all three types of bacteria. But, in regions where such preventative measures are not practiced, meningitis epidemics are a problem. In 1996, the largest meningitis epidemic ever recorded, in terms of the numbers of affected people, occurred in West Africa. An estimated 250,000 people contracted meningitis and 25,000 people died of the infection.

Leprosy is an example of a bacterial epidemic that used to be common and which is now on the way to being eliminated. The disease is caused by *Mycobacterium leprae*. The bacterium was discovered by G.A. Hansen in 1873, and was the first bacterium to be identified as a cause of human disease.

Epidemics of leprosy were common in ancient times; indeed, During the first century A.D., millions of people were afflicted with the disease. Nowadays, the number of leprosy patients in the entire world has been reduced some ninety percent over earlier times through a concerted campaign of diagnosis and treatment that began in the 1990s. Still, leprosy remains at epidemic proportions in six countries: Brazil, India, Madagascar, Mozambique, Myanmar, and Nepal. In these countries an estimated half million new cases of leprosy appear every year.

In contrast to leprosy, **tuberculosis** is an epidemic that is increasing in prevalence with time. Tuberculosis is caused by another mycobacterium called *Mycobacterium tuberculosis*. The lung infections caused by epidemics of tuberculosis kill two million people each year around the world. The number of cases of tuberculosis is growing because of the difficulty in supplying health care to some underdeveloped areas, the increase of immunocompromising diseases such as Human **Immunodeficiency** Syndrome, and the appearance and spread of a strain of the bacterium that is resistant to many of the drugs used to treat the infection. Estimates from the **World Health Organization** indicate that if the tuberculosis epidemic continues nearly one billion people will become infected by 2020. Of those, some 35 million people will die of tuberculosis.

The re-emergence of tuberculosis is paradoxical. Whereas other bacterial epidemics have and are being controlled by modern methods of treatment, such methods are exacerbating the tuberculosis epidemic. Part of this is also due to the target of the **bacterial infection**. Lung infections are harder to conquer than infections of the skin, such as occurs in leprosy. Moreover, when the **immune system** is not functioning properly, due to the presence of another infection, the lung infection can become deadly. Thus, tuberculosis is an example of a bacterial epidemic whose scope is changing with the emergence of other infections and treatments.

The tuberculosis epidemic also underscores the danger of ineffective treatment and the effect of modern life on the spread of disease. Poorly supervised and incomplete treatment has caused the emergence of the drug-resistant strains of the bacteria. The bacteria can remain in the lungs and so can infect others. With the greater movement of people around the globe, the spread of the disease by carriers increases.

A final example of a bacterial epidemic is cholera. The disease caused by *Vibrio cholerae* is an example of an ancient bacterial epidemic that continues today. The intestinal infection produces a watery diarrhea that can lead to a fatal dehydration. An epidemic of cholera caused by an antigenic version of *Vibrio cholerae* known as El Tor has been in progress since 1961. Indeed, the various epidemics are so widespread geographically that the disease can be considered pandemic (a simultaneously outbreak of illness on a worldwide scale). The latest epidemics have included countries in West Africa and Latin America that had been free of cholera for over a century.

Cholera is spread by contaminated food or water. Thus, the sanitary condition of a region is important to the presence of the epidemic. As with other bacterial epidemics of the past and present, underdeveloped regions are the focus of epidemic outbreaks of cholera.

See also Bacteria and bacterial infection; Biological warfare; Vaccination; Water quality

EPIDEMICS, VIRAL

An epidemic is an outbreak of a disease that involves a large number of people in a contained area (e.g., village, city, country). An epidemic that is worldwide in scope is referred to as a pandemic. A number of **viruses** have been responsible for **epidemics**. Some of these have been present since antiquity, while others have emerged only recently.

Smallpox is an example of an ancient viral epidemic. Outbreaks of smallpox were described in 1122 B.C. in China. In A.D. 165, Roman Legionnaires returning from military conquests in Asia and Africa spread the virus to Europe. One third of Europe's population died of smallpox during the 15-year epidemic. Smallpox remained a scourge until the late eighteenth century. Then, **Edward Jenner** devised a **vaccine** for the smallpox virus, based on the use of infected material from **cowpox** lesions. Less than a century later, naturally occurring smallpox epidemics had been ended.

Influenza is an example of a viral epidemic that also has its origins in ancient history. In contrast to smallpox, influenza epidemics remain a part of life today, even with the sophisticated medical care and vaccine development programs that can be brought to bear on infections.

Epidemics of influenza occurred in Europe during the Middle Ages. By the fifteenth century, epidemics began with regularity. A devastating epidemic swept through Spain, France, the Netherlands, and the British Isles in 1426–1427. Major outbreaks occurred in 1510, 1557, and 1580. In the eighteenth century there were three to five epidemics in Europe. Three more epidemics occurred in the nineteenth century. Another worldwide epidemic began in Europe in 1918. American soldiers returning home after World War I brought the virus to North America. In the United States alone almost 200,000 people died. The influenza epidemic of 1918 ranks as one of the worst natural disasters in history. In order to put the effects of the epidemic into perspective, the loss of life due to the four years of conflict of World War I was 10 million. The death toll from influenza during 5 months of the 1918 epidemic was 20 million.

Epidemics of influenza continue to occur. Examples include epidemics of the Asian flu (1957), and the Hong Kong

Clerks wearing cloth masks to avoid airborne contamination during an epidemic.

flu (1968). Potential epidemics due to the emergence of new forms of the virus in 1976 (the Swine flu) and 1977 (Russian flu) failed to materialize.

The continuing series of influenza epidemics is due to the ability of the various types of the influenza virus to alter the protein composition of their outer surface. Thus, the antibodies that result from an influenza epidemic in one year may be inadequate against the immunologically distinct influenza virus that occurs just a few years later. Advances in vaccine design and the use of agents that lessen the spread of the virus are contributing to a decreased scope of epidemics. Still, the threat of large scale influenza epidemics remains.

In the twentieth century, new viral epidemics have emerged. A number of different viruses have been grouped together under the designation of **hemorrhagic fevers**. These viruses are extremely contagious and sweep rapidly through the affected population. A hallmark of such infections is the copious internal bleeding that results from the viral destruction of host tissue. Death frequently occurs. The high death rate in fact limits the scope of these epidemics. Essentially the virus runs out of hosts to infect. The origin of hemorrhagic viruses such as the **Ebola virus** is unclear. A developing consensus is that the virus periodically crosses the species barrier from its natural pool in primates.

Another viral epidemic associated with the latter half of the twentieth century is acquired **immunodeficiency** syndrome. This debilitating and destructive disease of the **immune system** is almost certainly caused by several types of a virus referred to as the **Human Immunodeficiency Virus** (**HIV**). The first known death due to HIV infection was a man in the Congo in 1959. The virus was detected in the United States only in 1981. Subsequent examination of stored blood sample dating back 40 years earlier revealed the presence of HIV.

HIV may have arisen in Africa, either from a previously unknown virus, or by the mutation of a virus resident in a non-human population (e.g., primates). The tendency of the virus to establish a latent infection in the human host before the appearance of the symptoms of an active infection make it difficult to pinpoint the origin of the virus. Moreover, this aspect of latency, combined with the ready ability of man to travel the globe, contributes to the spread of the epidemic. Indeed, the epidemic may now be more accurately considered to be a pandemic.

A final example of a twentieth century viral epidemic is that caused by the Hanta virus. The virus causes a respiratory malady that can swiftly overwhelm and kill the patient. The virus is normally resident on certain species of mouse. In the mid-1990s, an epidemic of Hanta virus syndrome occurred in native populations in the Arizona and New Mexico areas of the United States west. As with other viral epidemics, the epidemic faded away as quickly as it had emerged. However, exposure of someone to the mouse host or to dried material containing the virus particles can just as quickly fuel another epidemic.

Given their history, it seems unlikely viral epidemics will be eliminated. While certain types of viral agents will be defeated, mainly by the development of effective vaccines and the undertaking of a worldwide **vaccination** program (e.g., smallpox), other viral diseases will continue to plague mankind.

See also AIDS; Hemorrhagic fevers and diseases; Virology

EPIDEMIOLOGY

Epidemiology is the study of the various factors that influence the occurrence, distribution, prevention, and control of disease, injury, and other health-related events in a defined human population. By the application of various analytical techniques including mathematical analysis of the data, the probable cause of an infectious outbreak can be pinpointed. This connection between epidemiology and infection makes **microorganisms** an important facet of epidemiology.

Epidemiology and genetics are two distinct disciplines that converge into a new field of human science. Genetic epidemiology, a broad term used for the study of genetics and inheritance of disease, is a science that deals with origin, distribution, and control of disease in groups of related individuals, as well as inherited causes of diseases in populations. In particular, genetic epidemiology focuses on the role of genetic factors and their interaction with environmental factors in the occurrence of disease. This area of epidemiology is also known as molecular epidemiology.

Much information can come from molecular epidemiology even in the exact genetic cause of the malady is not known. For example, the identification of a malady in generations of related people can trace the genetic characteristic, and even help identify the original source of the trait. This approach is commonly referred to as genetic screening. The knowledge of why a particular malady appears in certain people, or why such people are more prone to a microbial infection than other members of the population, can reveal much about the nature of the disease in the absence of the actual **gene** whose defect causes the disease.

Molecular epidemiology has been used to trace the cause of bacterial, viral, and parasitic diseases. This knowledge is valuable in developing a strategy to prevent further outbreaks of the microbial illness, since the probable source of a disease can be identified.

Furthermore, in the era of the use of biological weapons by individuals, organizations, and governments, epidemiological studies of the effect of exposure to infectious microbes has become more urgently important. Knowledge of the effect of a bioweapon on the battlefield may not extend to the civilian population that might also be secondarily affected by the weapons. Thus, epidemiology is an important tool in identifying and tracing the course of an infection.

The origin of a genetic disease, or the genetic defect that renders someone more susceptible to an infection (e.g., cystic fibrosis), can involve a single gene or can be more complex, involving more than one gene. The ability to sort through the information and the interplay of various environmental and genetic factors to approach an answer to the source of a disease outbreak, for example, requires sophisticated analytical tools and personnel.

Aided by advances in computer technology, scientists develop complex mathematical formulas for the analysis of genetic models, the description of the transmission of the disease, and genetic-environmental interactions. Sophisticated mathematical techniques are now used for assessing classification, diagnosis, prognosis and treatment of many genetic disorders. Strategies of analysis include population study and family study. Population study must be considered as a broad and reliable study with an impact on **public health** programs. They evaluate the distribution and the determinants of genetic traits. Family study approaches are more specific, and are usually confirmed by other independent observations. By means of several statistical tools, genetic epidemiologic studies evaluate risk factors, inheritance and possible models of inheritance. Different kinds of studies are based upon the number of people who participate and the method of sample collection (i.e., at the time of an outbreak or after an outbreak has occurred). A challenge for the investigator is to achieve a result able to be applied with as low a bias as possible to the general population. In other words, the goal of an epidemiological study of an infectious outbreak is to make the results from a few individuals applicable to the whole population.

Such analytical tools and trained personnel are associated more with the developed world, in the sense that expensive analytical equipment and chemicals, and highly trained personnel are required. However, efforts from the developed world have made such resources available to under-developed regions. For example, the response of agencies such as the **World Health Organization** to outbreaks of **hemorrhagic fevers** that occur in underdeveloped regions of Africa can include molecular epidemiologists.

A fundamental underpinning of infectious epidemiology is the confirmation that a disease outbreak has occurred. Once this is done, the disease is followed with time. The pattern of appearance of cases of the disease can be tracked by developing what is known as an epidemic curve. This information is vital in distinguishing a natural outbreak from a deliberate and hostile act, for example. In a natural outbreak the number of cases increases over time to a peak, after which the cases subside as **immunity** develops in the population. A deliberate release of organisms will be evident as a sudden appearance of a large number of cases at the same time.

Analysis of a proper sample size, as well as study type are techniques belonging to epidemiology and statistics. They were developed in order to produce reliable information from a study regarding the association of genetic and environmental factors. Studies that are more descriptive consider genetic trait frequency, geographic distribution differences, and prevalence of certain conditions in different populations. On the other hand, studies that analyze numerical data consider factors like association, probability of occurrence, inheritance, and identification of specific groups of individuals.

Thus, molecular epidemiology arises from varied scientific disciplines, including genetics, epidemiology, and statistics. The strategies involved in genetic epidemiology encompass population studies and family studies. Sophisticated mathematical tools are now involved, and computer technology is playing a predominant role in the development of the discipline. Multidisciplinary collaboration is crucial to understanding the role of genetic and environmental factors in disease processes.

See also Bacteria and bacterial infection; Genetic identification of microorganisms; History of microbiology; History of public health; Infection control; Public health, current issues; Transmission of pathogens

EPIDEMIOLOGY, TRACKING DISEASES WITH TECHNOLOGY

Epidemiology is a term that refers to the techniques and analysis methods that are used to pinpoint the source of an illness. As well, epidemiologists (those who conduct the epidemiological investigations) are concerned with the distribution of the infection.

Typically, epidemiology is concerned with an illness outbreak involving the sudden appearance of a disease or other malady among a group of people. Examples of situations where epidemiology would be of use are an outbreak of food poisoning among patrons of a restaurant, or a disease outbreak in a geographically confined area.

Many illnesses of epidemiological concern are caused by **microorganisms**. Examples include **hemorrhagic fevers** such as that caused by the **Ebola virus**, **toxic shock syndrome**, **Lyme disease** caused by the Norwalk virus, and Acquired **Immunodeficiency** Syndrome caused by the **Human Immunodeficiency Virus**. The determination of the nature of illness outbreaks due to these and other microorganisms involve microbiological and immunological techniques.

Various routes can spread infections (i.e., on contact, air borne, insect borne, food, water). Some microorganisms are spread via a certain route. For example, *Coxiella burnetii*, the cause of **Q fever**, is spread from animals to humans via the air. Knowledge of how an infection was spread can suggest possible causes of the infection. This saves time, since the elimination of the many infectious microorganisms requires a lot of laboratory analysis.

Likewise, the route of entry of an infectious microbe can also vary from microbe to microbe. Hepatitis viruses are transmitted via direct contact (e.g., sharing of needles). Thus, a water-borne illness is likely not due to a **hepatitis** virus.

If an outbreak is recognized early enough, samples of the suspected cause (i.e., food, in the case of a food poisoning incident) as well as samples from the afflicted (i.e., feces) can be gathered for analysis. The analysis will depend on the symptoms. For example, in the case of a food poisoning, symptoms such as the rapid development of cramping, nausea with vomiting, and diarrhea after eating a hamburger would be grounds to consider ***Escherichia coli*** O157:H7 as the culprit. Analyses would likely include the examination for other known microbes associated with food poisoning (i.e., ***Salmonella***) in order to save time in identifying the organism.

Analysis can involve the use of conventional laboratory techniques (e.g., use of nonselective and selective growth media to detect **bacteria**). As well, more recent technological innovations can be employed. An example is the use of antibodies to a known microorganism that are complexed with a fluorescent particle. The binding of the **antibody** to the microbes can be detected by the examination of a sample using fluorescence microscopy or flow cytometry. Molecular techniques such as the **polymerase chain reaction** are employed to detect genetic material from a target organism. However, the expense of the techniques such as **PCR** tend to limit its use to more of a confirmatory role, rather than as an initial tool of an investigation.

Another epidemiological tool is the determination of the antibiotic susceptibility and resistance of bacteria. This is especially true in the hospital setting, where **antibiotic resistance** bacteria are a problem in nosocomial (hospital acquired) infections. An outbreak of illness in a hospital should result in a pre-determined series of steps designed to rapidly determine the cause of the infection, to isolate the infection to as small an area of the hospital as possible, and to eliminate the infection. Knowing what **antibiotics** will be effective is a vital part of this strategy.

Such laboratory techniques can be combined with other techniques to provide information related to the spread of an outbreak. For example, microbiological data can be combined with geographic information systems (GIS). GIS information has helped pinpoint the source of outbreaks of Lyme disease. As well, the outbreak patterns can be used in the future to identify areas that will be high-risk areas for another outbreak. Besides geographic information, epidemiologists will use information including the weather on the days preceding an outbreak, mass transit travel schedules and schedules of mass-participation events that occurred around the time of an outbreak to try an establish a pattern of movement or behavior to those who have been affected by the outbreak. Use of credit cards and bank debit cards can also help piece together the movements of those who subsequently became infected.

The spread of **AIDS** in North America provides an example of the result of an epidemiological study of an illness. Analysis of the pattern of outbreaks and tracing the behavioral patterns of those who became infected led to the conclusion that the likely originator of the epidemic was a flight atten-

dant. Because of his work, he was well traveled. His sexual behavior helped spread the virus to sexual partners all over North America, and they subsequently passed the virus on to other partners. Without the techniques and investigative protocols of epidemiology, the source of the AIDS epidemic would not have been resolved.

Reconstructing the movements of people is especially important when the outbreak is of an infectious disease. The occurrence of the disease over time can yield information as to the source of an outbreak. For example, the appearance of a few cases at first with the number of cases increasing over time to a peak is indicative of a natural outbreak. The number of cases usually begins to subside as the population develops **immunity** to the infection (e.g., **influenza**). However, if a large number of cases occur in the same area at the same time, the source of the infection might not be natural. Examples include a food poisoning or a bioterrorist action.

The ultimate aim of the various steps taken in an epidemiological investigation is to prevent infections by the use of prudent **public health** measures, rather than having to rely on reactive steps such as **vaccination** to defeat ongoing infections. Indeed, for some infections (i.e., **HIV**, hepatitis B and C) vaccination may not ultimately prove to be as effective as the identification of the factors that promote the diseases, and addressing those factors.

See also Bacteria and bacterial infection; Epidemics and pandemics; Laboratory techniques in immunology; Laboratory techniques in microbiology

EPIDERMAL INFECTIONS • *see* SKIN INFECTIONS

EPISOMES, PLASMIDS, INSERTION SEQUENCES, AND TRANSPOSONS

Episomes, **plasmids**, insertion sequences, and transposons are elements of **DNA** (**deoxyribonucleic acid**) that can exist independent of the main, or genomic, DNA.

An episome is a non-essential genetic element. In addition to its independent existence, an episome can also exist as an integrated part of the host genome of **bacteria**. It originates outside the host, in a virus or another bacterium. When integrated, a new copy of the episome will be made as the host chromosome undergoes replication. As an autonomous unit, the viral episome genetic material destroys the host cell as it utilizes the cellular replication machinery to make new copies of itself. But, when integrated into the bacterial chromosome they multiply in cell division and are transferred to the daughter cells. Another type of episome is called the F factor. The F factor is the best studied of the incompatibility groups that have the property of **conjugation** (the transfer of genetic material from one bacterial cell to another). The F factor can exist in three states. F+ is the autonomous, extrachromosomal state. Hfr (or high frequency **recombination**) refers to a factor, which has integrated into the host chromosome. Finally, F, or F prime, state refers to the factor when it exists outside the chromosome, but with a section of chromosomal DNA attached to it. An episome is distinguished from other pieces of extrachromosomal DNA, such as plasmids, on the basis of their size. Episomes are large, having a molecular weight of at least 62 kilobases.

In contrast to episomes, a plasmid exists only as an independent piece of DNA. It is not capable of integration with the chromosomal DNA; it carries all the information necessary for its own replication. In order to maintain itself, a plasmid must divide at the same rate as the host bacterium. A plasmid is typically smaller than an episome, and exists as a closed circular piece of double stranded DNA. A plasmid can be readily distinguished from the chromosomal DNA by the techniques of gel **electrophoresis** or cesium chloride buoyant density gradient centrifugation. In addition to the information necessary for their replication, a plasmid can carry virtually any other **gene**. While not necessary for bacterial survival, plasmids can convey a selective advantage on the host bacterium. For example, some plasmids carry genes encoding resistance to certain **antibiotics**. Such plasmids are termed resistance or R factors. Other traits carried on plasmids include degradation of complex macromolecules, production of bacteriocins (molecules that inhibit **bacterial growth** or kill the bacteria), resistance to various heavy metals, or disease-causing factors necessary for infection of animal or plant hosts. Such traits can then be passed on to other bacteria, as some (but not all) plasmids also have the ability to promote transfer of their genetic material, in a process called conjugation. Conjugation is a one-way event—the DNA is transferred from one bacterium (the donor) to another bacterium (the recipient). All plasmids belong to one of the 30 or more incompatibility groups. The groups determine which plasmids can co-exist in a bacterial cell and help ensure that the optimum number of copies of each plasmid is maintained.

Plasmids have been exploited in **molecular biology** research. The incorporation of genes into plasmids, which maintain large numbers of copies in a cell (so-called multicopy plasmids), allows higher levels of the gene product to be expressed. Such plasmids are also a good source of DNA for **cloning**.

Transposons and insertion sequences are known as mobile genetic elements. While they can also exist outside of the chromosome, they prefer and are designed to integrate into the chromosome following their movement from one cell to another. The are of interest to researchers for the insight they provide into basic molecular biology and **evolution**, as well as for their use as basic genetic tools. Transposons contain genes unrelated to the **transposition** of the genetic material from one cell to another. For example, Class 1 transposons encode drug resistance genes. In contrast, insertion sequences encode only the functions involved in their insertion into chromosomal DNA. Both transposons and insertion sequences can induce changes in chromosomal DNA upon their exiting and insertions, and so can generate **mutations**.

See also Bacteria; DNA (deoxyribonucleic acid); Electro-phoresis; Microbial genetics

EPSTEIN-BARR VIRUS

Epstein-Barr virus (EBV) is part of the family of human **herpes viruses**. Infectious **mononucleosis** (IM) is the most common disease manifestation of this virus, which once established in the host, can never be completely eradicated. Very little can be done to treat EBV; most methods can only alleviate resultant symptoms.

In addition to infectious mononucleosis, EBV has also been identified in association with—although not necessarily believed to cause—as many as 50 different illnesses and diseases, including chronic fatigue syndrome, rheumatoid arthritis, arthralgia (joint pain without **inflammation**), and myalgia (muscle pain). While studying aplastic anemia (failure of bone marrow to produce sufficient red blood cells), researchers identified EBV in bone marrow cells of some patients, suggesting the virus may be one causative agent in the disease. Also, several types of cancer can be linked to presence of EBV, particularly in those with suppressed immune systems, for example, suffering from **AIDS** or having recently undergone kidney or liver transplantation. The diseases include hairy cell leukemia, Hodgkin's and non-Hodgkin lymphoma, Burkitt's lymphoma (cancer of the lymphatic system endemic to populations in Africa), and nasopharyngeal carcinoma (cancers of the nose, throat, and thymus gland, particularly prevalent in East Asia). Recently, EBV has been associated with malignant smooth-muscle tissue tumors in immunocompromised children. Such tumors were found in several children with AIDS and some who had received liver transplants. Conversely, it appears that immunosuppressed adults show no elevated rates of these tumors.

Epstein-Barr virus was first discovered in 1964 by three researchers—Epstein, Achong, and Barr—while studying a form of cancer prevalent in Africa called Burkitt's lymphoma. Later, its role in IM was identified. A surge of interest in the virus has now determined that up to 95% of all adults have been infected with EBV at some stage of their lives. In seriously immunocompromised individuals and those with inherited **immune system** deficiencies, the virus can become chronic, resulting in "chronic Epstein-Barr virus" which can be fatal.

EBV is restricted to a very few cells in the host. Initially, the infection begins with its occupation and replication in the thin layer of tissue lining the mouth, throat, and cervix, which allow viral replication. The virus then invades the **B cells**, which do not facilitate the virus's replication but do permit its occupation. Infected B cells may lie dormant for long periods or start rapidly producing new cells. Once activated in this way, the B cells often produce antibodies against the virus residing in them. EBV is controlled and contained by killer cells and suppressor cells known as CD4 T lymphocytes in the immune system. Later, certain cytotoxic (destructive) CD8 T lymphocytes with specific action against EBV also come into play. These cells normally defend the host against the spread of EBV for the life of the host.

A healthy body usually provides effective **immunity** to EBV in the form of several different antibodies, but when this natural defense mechanism is weakened by factors that suppress its normal functioning—factors such as AIDS, organ transplantation, bone marrow failure, **chemotherapy** and other drugs used to treat malignancies, or even extended periods of lack of sleep and overexertion—EBV escape from their homes in the B cells, disseminate to other bodily tissue, and manifest in disease.

Infection is determined by testing for the antibodies produced by the immune system to fight the virus. The level of a particular antibody—the heterophile antibody—in the blood stream is a good indicator of the intensity and stage of EBV infection. Even though EBV proliferates in the mouth and throat, cultures taken from that area to determine infection are time-consuming, cumbersome, and usually not accurate.

Spread of the virus from one person to another requires close contact. Because of viral proliferation and replication in the lining of the mouth, infectious mononucleosis is often dubbed "the kissing disease." Also, because it inhabits cervical cells, researchers now suspect EBV may be sexually transmitted. Rarely is EBV transmitted via blood transfusion.

EBV is one of the latent viruses, which means it may be present in the body, lying dormant often for many years and manifesting no symptoms of disease. The percentage of shedding (transmission) of the virus from the mouth is highest in people with active IM or who have become immunocompromised for other reasons. A person with active IM can prevent transmission of the disease by avoiding direct contact—such as kissing—with uninfected people. However, shedding has been found to occur in 15% of adults who test positive for antibodies but who show no other signs of infection, thus allowing the virus to be transmitted. Research efforts are directed at finding a suitable **vaccine**.

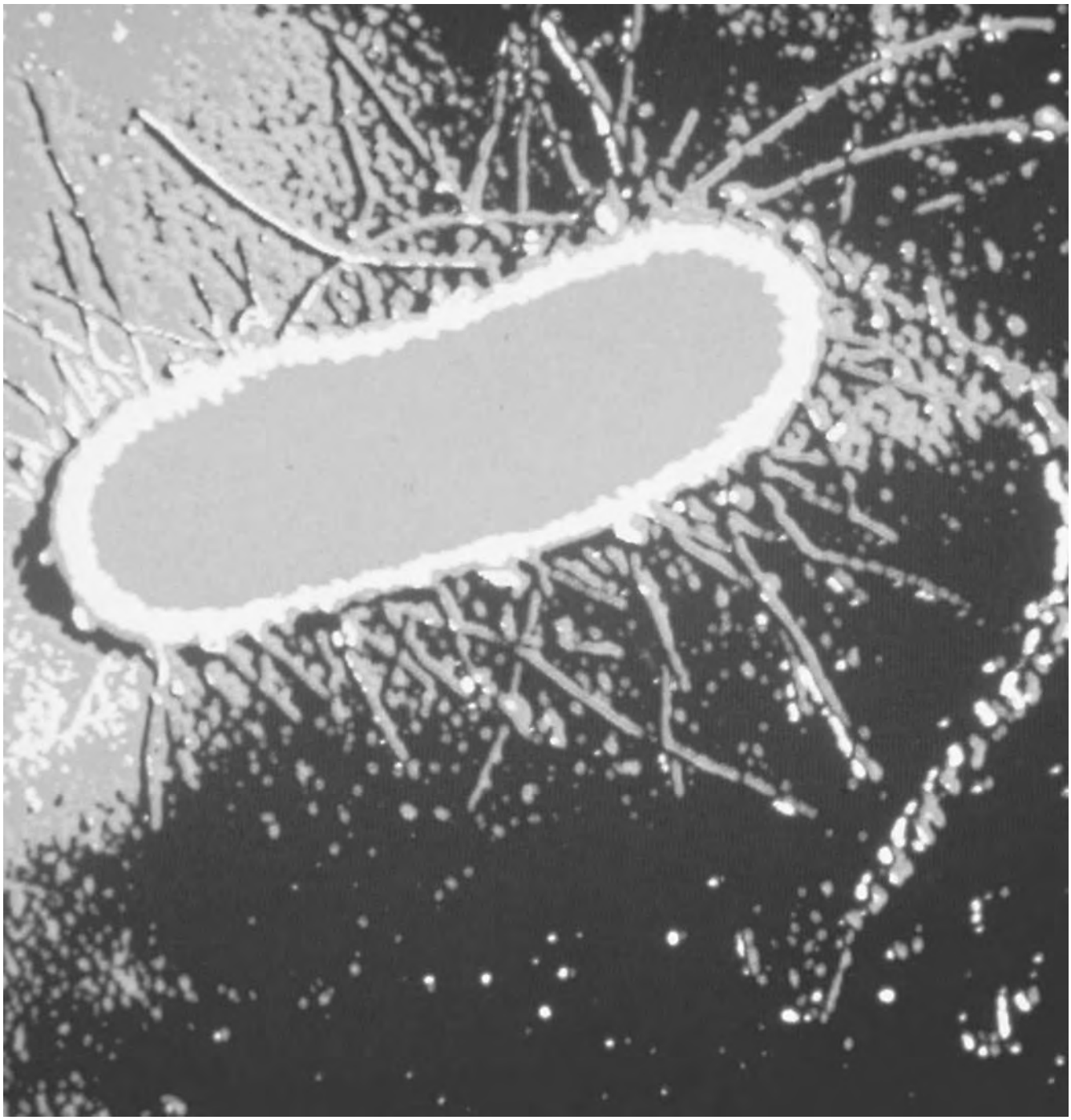
The prevalence of antibodies against EBV in the general population is high in developing countries and lower socioeconomic groups where individuals become exposed to the virus at a very young age. In developed countries, such as the United States, only 50% of the population shows traces of **antibody** by the age of five years, with an additional 12% in college-aged adolescents, half of whom will actually develop IM. This situation indicates that children and young persons between the ages of 10 and 21 years are highly susceptible to IM in developed countries, making it a significant health problem among students.

See also Latent viruses and diseases; Mononucleosis, infectious; Viruses and responses to viral infection

ERYTHROMYCINS • *see* ANTIBIOTICS

ESCHERICHIA COLI

Escherichia coli is a bacterium, which inhabits the intestinal tract of humans and other warm-blooded mammals. It constitutes approximately 0.1% of the total **bacteria** in the adult intestinal tract. Its name comes from the name of the person, Escherich, who in 1885 first isolated and characterized the bacterium.



Scanning electron micrograph of an *Escherichia coli* bacterium.

The bacterium, particularly one type called strain K–12, is one of the most widely studied organisms in modern research. Its biochemical behavior and structure are well known, having been studied for much of this century. This plethora of information has made *E. coli* indispensable as the bacterial model system for biochemical, behavioral and structural studies. *E. coli* is also the most encountered bacterium in clinical laboratories, being the primary cause of human urinary tract infections. Pathogenic (diseases causing) strains of *E. coli* are responsible for **pneumonia**, **meningitis** and traveler's diarrhea.

As part of the normal flora of the intestinal tract, *E. coli* is beneficial. It is crucial in the digestion of food, and is our principle source of vitamin K and B-complex vitamins.

Outside of the intestinal tract, *E. coli* dies quickly. This trait has been exploited, and *E. coli* is a popular indicator of drinking **water quality**, as its presence indicates the recent **contamination** of the water with feces.

One of the most harmful types of *E. coli* is a strain called O157:H7. Researchers surmise that O157:H7 arose when an innocuous *E. coli* bacterium was infected by a virus carrying the genes coding for a powerful toxin called Shiga-like toxin. The toxin can destroy cells in the intestinal tract and, if they enter the bloodstream, can impair or destroy the kidneys and the liver. The intestinal damage causes a lot of bleeding. In children and elderly people, this hemorrhaging can be lethal. In other people, damage to the kidney and liver can be permanent or even lethal. In the summer of 2000, more than 2,000 people in Walkerton, Ontario, Canada were sickened and seven people died from drinking water which had been contaminated with O157:H7.

Strain O157:H7 was first linked to human disease in 1983, when it was shown to have been the cause of two outbreaks of an unusual and severe gastrointestinal ailment in the Unites States. Since then, the number of documented human illnesses and deaths caused by O157:H7 has increased steadily worldwide. Disease caused by *E. coli* is preventable, by proper hand washing after bowel movements, avoidance of unpasteurized milk or apple cider, washing of raw foods before consumption and thorough cooking of ground meat.

Modern genetics techniques have been successful in obtaining the sequence of the genetic material of *E. coli*. Frederick Blattner and his colleagues published the genome sequence of strain K–12 in 1997. The genome was discovered to have approximately 4300 protein coding regions making up about 88 per cent of the bacterial chromosome. The most numerous types of proteins were transport and binding proteins—those necessary for the intake of nutrients. A fairly large portion of the genome is reserved for metabolism—the processing of the acquired nutrients into useable chemicals. In 2000, Nicole Perna and her colleagues published the genome sequence of O157:H7. The O157:H7 genome shows similarity to tat of k12, reflecting a common ancestry. But, in contrast to K12, much of the genome of O157:H7 codes for unique proteins, over 1,300, some of which may be involved in disease causing traits. Many of these genes appear to have been acquired from other **microorganisms**, in a process called lateral transfer. Thus, strain O157:H7 appears to be designed to undergo rapid genetic change. This distinction is important; indicating that strategies to combat problems caused by one strain of *E. coli* might not be universally successful. Knowledge of the genetic organization of these strains will enable more selective strategies to be developed to combat *E.coli* infections.

See also Food safety; Microbial flora of the stomach and gastrointestinal tract; Microbial genetics; Waste water treatment; Water purification; Water quality

ESCHERICHIA COLI (E. COLI) INFECTION

• *see* E. COLI O157:H7 INFECTION

ESCHERICHIA COLI, ENTEROHEMORRHAGIC • *see* ESCHERICHIA COLI (E. COLI)

EUBACTERIA

The Eubacteria are the largest and most diverse taxonomic group of **bacteria**. Some scientists regard the Eubacteria group as an artificial assemblage, merely a group of convenience rather than a natural grouping. Other scientists regard eubacteria as comprising their own kingdom. Another recent classification holds Eubacteria and Archaebacteria as domains or major groupings, classified above the kingdom level. The Eubacteria are all easily stained, rod-shaped or spherical bacteria. They are generally unicellular, but a small number of multicellular forms do occur. They can be motile or nonmotile and the motile forms are frequently characterized by the presence of numerous flagellae. Many of the ecologically important bacteria responsible for the fixation of nitrogen, such as *Azotobacter* and *Rhizobium,* are found in this group.

The cell walls of all of these species are relatively thick and unchanging, thus shape is generally constant within groups found in the Eubacteria. Thick cell walls are an evolutionary adaptation that allows survival in extreme situations where thinner walled bacteria would dry out. Some of the bacteria are gram positive while others are gram negative. One commonality that can be found within the group is that they all reproduce by transverse binary fission, although not all bacteria that reproduce in this manner are members of this group.

Eubacteria are often classified according to the manner in which they gain energy. Photoautotrophic Eubacteria manufacture their own energy through **photosynthesis**. Cyanobacteria, often called **blue-green algae**, are common photoautotrophic Eubacteria that are found in ponds and wetlands. Although not true algae, Cyanobacteria grow in chain-like colonies and contain chloroplasts as do aquatic algae. Cyanobacteria fossils are among the oldest-known fossils on Earth, some more than 3.5 billion years old.

Heterotrphic Eubacteria depend upon organic molecules to provide a source of energy. Heterotrophic Eubacteria are among the most abundant and diverse bacteria on Earth, and include bacteria that live as **parasites**, decomposers of organic material (saprophytes), as well as many pathogens (disease-causing bacteria). Chemoautotrophic Eubacteria bacteria obtain their own energy by the oxidation of inorganic molecules. **Chemoautotrophic bacteria** are responsible for releasing the sulfur resulting in a sulfur taste of freshwater near many beaches (such as in Florida), and for supplying nitrogen in a form able to be used by plants.

See also Autotrophic bacteria; Heterotrophic bacteria; Nitrogen cycle in microorganisms; Oxidation-reduction reaction; Photosynthetic microorganisms

EUKARYOTES

Eukaryotic organisms encompass a range of organisms, from humans to single-celled **microorganisms** such as **protozoa**. Eukaryotes are fundamentally different from prokaryotic microorganisms, such as **bacteria**, in their size, structure and functional organization.

The oldest known eukaryote fossil is about 1.5 billion years old. Prokaryote fossils that are over 3 billion years old are known. Thus, prokaryotic cells appeared first on Earth. The appearance of eukaryotic cells some 1.5 billion years ago became possible when cellular function was organized into regions within the cell called organelles.

The eukaryotes are organized into a division of life that is designated as the Eukaryota. The Eukaryota are one of the three branches of living organisms. The other two branches are the Prokaryota and the Archae.

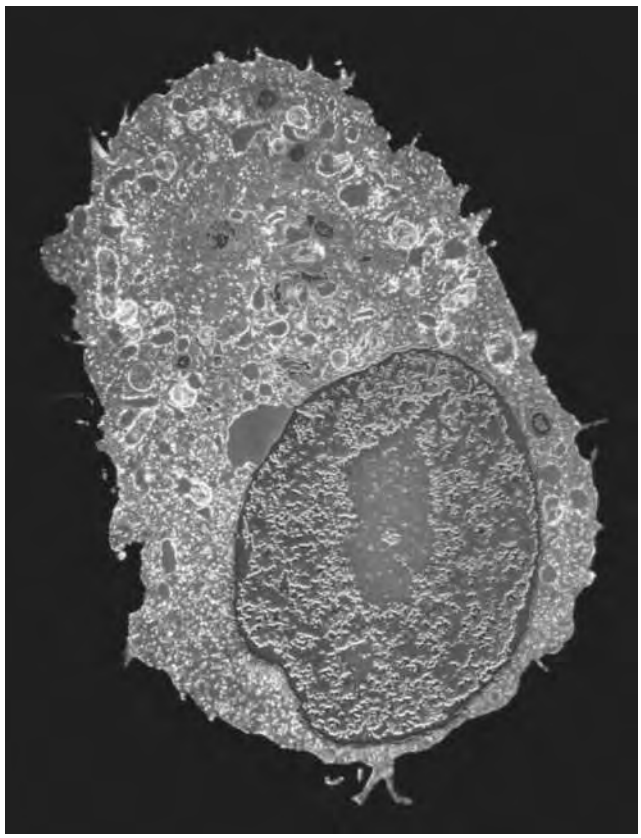
The evolutionary divergence of life into these three groups has been deduced in the pasts several decades. Techniques of molecular analysis have been used, in particular the analysis of the sequence of a component of ribosomal **ribonucleic acid** (**RNA**), which is known as 16S RNA. This RNA species is highly conserved in life forms. Thus, great differences in the sequence of 16 S RNA between a eukaryotic and a prokaryotic microorganism, for example, indicate that the two organisms diverged evolutionarily a very long time ago. A similar 16 S RNA indicates the converse; that evolutionary branching is a relatively recent event.

Eukaryotic cells are about 10 times the size of all but a few prokaryotes. This translates to an internal volume which is very much larger, some 1000 times, that the internal volume of a bacterium. In order to survive, eukaryotes evolved a highly organized internal structure, in order that all the tasks necessary for life can be accomplished in the large internal volume. This internal structure is the fundamental distinguishing aspect of a eukaryote versus a prokaryote.

Functional specialization is the fundamental hallmark of eukaryotes. In larger organisms, such as humans, this specialization gives rise to organs such as the heart, lover, and brain, and to functional organizations such as the **immune system**. But organization is also evident in microscopic, even single-celled, eukaryotes.

In a eukaryote, the nuclear material is segregated within a specialized region called the **nucleus**. This feature is a key constituent of eukaryotic cells. Indeed, the word eukaryote means “true nuclei.” The nucleus exists because of the presence of the so-called nuclear membrane, which encloses the nuclear material. The nuclear membrane contains pores, through which material can enter and leave the nuclear region. Prokaryotes lack an organized nucleus. Indeed, for many years the presence of a nucleus was the sole key feature that distinguished a eukaryote from a prokaryote.

Most of the eukaryotic **DNA** (**deoxyribonucleic acid**) is present in the nucleus. The remainder is contained within the energy-generating structures known as the mitochondria. The organization of the eukaryotic DNA is very different from bacterial DNA. In the latter, the genetic material is usually dispersed as a large circle throughout the interior of the bacterium, in a gel-like mixture termed the **cytoplasm**. In contrast, eukaryotic DNA is organized into discrete limb-like structures called **chromosomes**.



A eukaryotic cell.

The replication of eukaryotic DNA is also different from that of prokaryotes. The latter is essentially an unwinding of the double helix of DNA, with ongoing complementary copies of daughter DNA strands made from each unwinding parental strand. The result is two double helices. The replication process in eukaryotes is more complex, involving several phases of chromosome replication, segregation to areas of the cell, collection together, and enclosure in a nuclear membrane.

Eukaryotic cells, including microorganisms, contain a specialized functional region known as the endoplasmic reticulum. This network of tubular structures is involved in the manufacture of protein from the template of RNA. In many eukaryotes a region called the Golgi apparatus or Golgi body is associated with the endoplasmic reticulum. The Golgi body is involved with the transport of compounds into and out of the cell.

Another distinctive feature of eukaryotic cells is the aforementioned mitochondria. These are the energy factories of the cell. Additionally, some eukaryotes possess structures called chloroplasts, which use the energy available in light to change carbon dioxide and water into carbohydrates. The carbohydrates provide a ready source of energy for cellular func-

tions. This photosynthetic process is a feature of the microscopic eukaryotes called algae.

Other internal organization of eukaryotes includes lysosomes, which contain enzyme that digest food that is taken into the eukaryote. The **lysosome** represents a primitive stomach.

Eukaryotic cells such as amoeba possess an internal scaffolding that helps provide the shape and support to the cell. The scaffolding consists of filaments that are made of protein. Depending on the protein the filaments are designated as actin filaments, microtubules, and intermediate filaments.

Eukaryotes such as amoebae and algae are part of a group that is called Protista. More commonly, members of the group are referred to as **protists**.

The evolutionary branching of eukaryotes from prokaryotes involved the acquisition of regions specialized function within the eukaryotic cell. One of these regions, the mitochondria, was likely derived from the habitation of a eukaryote by a bacterium. Evidence from ultrastructural and molecular studies for a symbiosis between a bacterium and a eukaryote is convincing. Over time, the bacterium became truly a component of the eukaryotic cell. Today, however, the DNA of the mitochondria remains unique, with respect to eukaryotic nuclear DNA.

Likewise, chloroplasts may have had the origin in a symbiotic relationship between a cyanobacterium and a eukaryotic cell. Current evidence does not support the development of any other eukaryotic organelle from a prokaryotic ancestor.

See also Bacterial ultrastructure; Cell cycle and cell division; Mitochondrial DNA

EUKARYOTIC CELLS, GENETIC REGULATION OF • *see* GENETIC REGULATION OF EUKARYOTIC CELLS

EULER-CHELPIN, HANS VON (1873-1964)

Swedish biochemist

Hans von Euler-Chelpin described the role of **enzymes** in the process of **fermentation** and also researched vitamins, tumors, enzymes, and coenzymes. He was an important contributor in the discovery of the structure of certain vitamins. In 1929, he shared the Nobel Prize in chemistry with Arthur Harden for their research on the fermentation of sugar and enzymes. Euler-Chelpin's research has far-reaching implications in the fields of nutrition and medicine.

Hans Karl Simon August von Euler-Chelpin was born in Augsburg in the Bavarian region of Germany on February 15, 1873, to Rigas, a captain in the Royal Bavarian Regiment, and Gabrielle (Furtner) von Euler-Chelpin. His mother was related to the Swiss mathematician Leonhard Euler. Shortly after his birth, Euler-Chelpin's father was transferred to Munich and Euler-Chelpin lived with his grandmother in Wasserburg for a time. After his early education in Munich, Würzburg, and Ulm, he entered the Munich Academy of Painting in 1891 intending to become an artist. Eventually, he changed his professional interest to science.

In 1893, Euler-Chelpin enrolled at the University of Munich to study physics with Max Planck and Emil Warburg. He also studied organic chemistry with Emil Fischer and A. Rosenheim, after which he worked with Walther Nernst at the University of Göttingen on problems in physical chemistry. This post-doctoral work in the years 1896 to 1897 was undertaken after Euler-Chelpin received his doctorate in 1895 from the University of Berlin.

The summer of 1897 was the first of several that Euler-Chelpin spent in apprentice roles in Stockholm and in Berlin. He served as an assistant to **Svante Arrhenius** in his laboratory at the University of Stockholm, becoming a privatdocent (unpaid tutor) there in 1899. Returning to Germany that summer, he studied with Eduard Buchner and Jacobus Van't Hoff in Berlin until 1900. His studies during this period centered on physical chemistry, which was receiving a great deal of attention at that time in both Germany and Sweden. Recognition came early to Euler-Chelpin for his work. He received the Lindblom Prize from Germany in 1898.

It was evident during this time that there were new opportunities in organic chemistry. The new equipment used to measure properties could be applied to the complexities of chemical changes that took place in organisms. Euler-Chelpin's interests, therefore, shifted to organic chemistry. He visited the laboratories of others working in the field, such as Arthur Hantzsch and Johannes Thiele in Germany and G. Bertrand in Paris. These contacts contributed to his developing interest in fermentation.

In 1902, Euler-Chelpin became a Swedish citizen and in 1906, he was appointed professor of general and organic chemistry at the University of Stockholm, where he remained until his retirement in 1941. By 1910, Euler-Chelpin was able to present the fermentation process and enzyme chemistry into a systematic relationship with existing chemical knowledge. His book, *The Chemistry of Enzymes,* was first published in 1910 and again in several later editions.

In spite of being a Swedish citizen, Euler-Chelpin served in the German army during World War I, fulfilling his teaching obligations for six months of the year and military service for the remaining six. In the winter of 1916–1917, he took part in a mission to Turkey, a German ally during World War I, to accelerate the production of munitions and alcohol. He also commanded a bomber squadron at the end of the war.

After the war, Euler-Chelpin began his research into the chemistry of enzymes, particularly in the role they played in the fermentation process. This study was important because enzymes are the catalysts for biochemical reactions in plant and animal organisms. An integral aspect of Euler-Chelpin's work with enzymes was to identify each substrate (the molecule upon which an enzyme acted) in the reaction. He succeeded in demonstrating that two fragments that split from the sugar molecule were disparate in energy. He further illustrated that the less energetic fragment, which is attached to the phosphate, is destroyed in the process. Apart from tracing the phosphate through the fermentation sequence, Euler-Chelpin

detailed the chemical makeup of cozymase, a non-protein constituent involved in cellular **respiration**.

In 1929, Euler-Chelpin was awarded the Nobel Prize in chemistry, which he shared with Arthur Harden "for their investigations on the fermentation of sugar and of fermentative enzymes." The presenter of the award noted that fermentation was "one of the most complicated and difficult problems of chemical research." The solution to the problem made it possible, the presenter continued, "to draw important conclusions concerning carbohydrate **metabolism** in general in both the vegetable and the animal organism."

In 1929, Euler-Chelpin became the director of the Vitamin Institute and Institute of **Biochemistry** at the University of Stockholm, which was founded jointly by the Wallenburg Foundation and the Rockefeller Foundation. Although he retired from teaching in 1941, he continued research for the remainder of his life. In 1935, he had turned his attention to the biochemistry of tumors and developed, through his collaboration with George de Hevesy, a technique for labeling the nucleic acids present in tumors, which subsequently made it possible to trace their behavior. He also helped elucidate the function of nicotinamide and thiamine in compounds which are metabolically active.

Euler-Chelpin was twice married, each time to a woman who assisted him in his research. His first wife, Astrid Cleve, was the daughter of P. T. Cleve, a professor of chemistry at the University of Uppsala. She helped him in his early research in fermentation. They married in 1902, had five children, and divorced in 1912. Euler-Chelpin married Elisabeth, Baroness Ugglas in 1913, with whom he had four children. This marriage lasted for fifty-one years. A son by his first wife, Ulf Euler, later also won a Nobel Prize. His award was made in 1970 in the field of medicine or physiology for his work on neurotransmitters and the nervous system.

Euler-Chelpin was awarded the Grand Cross for Federal Services with Star from Germany in 1959. He also received numerous honorary degrees from universities in Europe and America. He held memberships in Swedish science associations, as well as many foreign professional societies. He is the author of more than eleven hundred research papers and over half a dozen books. Euler-Chelpin died on November 6, 1964, in Stockholm, Sweden.

EVANS, ALICE (1881-1975)

American microbiologist

The bacteriologist Alice Evans was a pioneer both as a scientist and as a woman. Evans discovered that the *Brucella* **bacteria**, contracted from farm animals and their milk, was the cause of undulant fever in humans, and responded by fighting persistently for the routine, improved **pasteurization** of milk, eventually achieving success. She was the first woman president of the Society of American Bacteriologists (now American Society of Microbiology). Although marginalized early in her career, Evans overcame many obstacles and lived to see her discoveries repeatedly confirmed. She had a major impact on microbiology in the United States and the world, and received belated honors for her numerous achievements in the field.

Alice Catherine Evans was born on January 29, 1881, in the predominantly Welsh town of Neath, Pennsylvania, the second of William Howell and Anne Evans' two children. William Howell, the son of a Welshman, was a surveyor, teacher, farmer, and Civil War veteran. Anne Evans, also Welsh, emigrated from Wales at the age of 14. Evans received her primary education at the local district school. She went on to study at the Susquehanna Institute at Towanda, Pennsylvania. She wished to go to college but, unable to afford tuition, took a post as a grade school teacher. After teaching for four years, she enrolled in a tuition-free, two-year course in nature study at the Cornell University College of Agriculture. The course was designed to help teachers in rural areas inspire an appreciation of nature in their students. It changed the path of Evans' life, however, and she never returned to the schoolroom.

At Cornell, Evans discovered her love of science and received a B.S. degree in agriculture. She chose to pursue an advanced degree in bacteriology and was recommended by her professor at Cornell for a scholarship at the University of Wisconsin. She was the first woman to receive the scholarship, and under the supervision of E. G. Hastings, Evans studied bacteriology with a focus on chemistry. In 1910, she received a Master of Science degree in bacteriology from Wisconsin. Although encouraged to pursue a Ph.D., Evans accepted a research position with the University of Wisconsin Agriculture Department's Dairy Division and began researching cheesemaking methods in 1911. In 1913, she moved with the division to Washington, D.C., and served as bacteriological technician in a team effort to isolate the sources of **contamination** of raw cow's milk, which were then assumed to be external.

On her own, Evans began to focus on the intrinsic bacteria in raw cow's milk. By 1917, she had found that the bacterium responsible for undulant or "Malta" fever (later called **brucellosis**, after the responsible pathogen) was similar in important respects to one associated with spontaneous abortions in cows, and that the two bacteria produced similar clinical effects in guinea pigs. Prevailing wisdom at the time held that many bovine diseases could not be transmitted to humans. That year she presented her findings to the Society of American Bacteriologists; her ideas were received with skepticism that may have been more due to her gender and level of education than her data.

In 1918, Evans was asked to join the staff of the United States Public Health Service by director George McCoy. There, she was absorbed in the study of **meningitis**. Although she was unable to continue her milk studies during this time, support for Evans' findings was trickling in from all over the world. By the early 1920s, it was recognized that undulant fever and Malta fever were due to the same bacteria, but there was still resistance to the idea that humans could contract brucellosis by drinking the milk of infected cows. Because the symptoms of brucellosis were so similar to those of **influenza**, **typhoid fever**, **tuberculosis**, **malaria**, and rheumatism, it was not often correctly diagnosed. Evans began documenting cases of the disease among humans in the U.S. and South Africa, but

it was not until 1930, after brucellosis had claimed the lives of a number of farmers' children in the U.S., that **public health** officials began to recognize the need for pasteurization.

In 1922, Evans, like many others who researched these organisms, became ill with brucellosis. Her condition was chronic, plaguing her on and off for almost 23 years, and perhaps providing her with new insight into the disease. As the problem of chronic illness became widespread, Evans began surveying different parts of the U.S. to determine the numbers of infected cows from whom raw milk was sold, and the numbers of chronic cases resulting from the milk.

In 1925, Evans was asked to serve on the National Research Council's Committee on Infectious Abortion. In this capacity, Evans argued for the pasteurization of milk, a practice that later became an industry standard. In recognition of her achievements, Evans was in 1928 elected the first woman president of the American Society of Bacteriologists. In 1930, she was chosen, along with Robert E. Buchanan of Iowa State University, as an American delegate to the First International Congress of Bacteriology in Paris. She attended the second Congress in London in 1936 and was again able to travel widely in Europe. She returned to the United States and eventually was promoted to senior bacteriologist at the Public Health Service, by then called the National Institute of Health. By 1939, Evans had changed her focus to **immunity** to streptococcal infections and in 1945, she retired. Evans, who never married, died at the age of 94 on September 5, 1975, in Alexandria, Virginia.

Evolution and evolutionary mechanisms

Evolution is the process of biological change over time. Such changes, especially at the genetic level are accomplished by a complex set of evolutionary mechanisms that act to increase or decrease genetic variation. Because of their rapid development and reproduction (i.e., high generation rate), evidence of the fundamental molecular mechanisms of evolution are especially apparent in studies of **bacteria** and viral **microorganisms**. Immunological adaptation has a profound effect on fitness and survivability.

Evolutionary theory is the cornerstone of modern biology, and unites all the fields of biology under one theoretical umbrella to explain the changes in any given **gene** pool of a population over time. Evolutionary theory is theory in the scientific usage of the word. It is more than a hypothesis; there is an abundance of observational and experimental data to support the theory and its subtle variations. These variations in the interpretation of the role of various evolutionary mechanisms are because all theories, no matter how highly useful or cherished, are subject to being discarded or modified when verifiable data demand such revision. Biological evolutionary theory is compatible with nucleosynthesis (the evolution of the elements) and current cosmological theories in physics regarding the origin and evolution of the Universe. There is no currently accepted scientific data that is incompatible with the general postulates of evolutionary theory, and the mechanisms of evolution.

Fundamental to the concept of evolutionary mechanism is the concept of the syngameon, the set of all genes. By definition, a gene is a hereditary unit in the syngameon that carries information that can be used to construct proteins via the processes of **transcription** and **translation**. A gene pool is the set of all genes in a species or population.

Another essential concept, important to understanding evolutionary mechanisms, is an understanding that there are no existing (extant) primitive organisms that can be used to study evolutionary mechanism. For example, all **eukaryotes** derived from a primitive, common prokaryotic ancestral bacterium. Accordingly, all living eukaryotes have evolved as eukaryotes for the same amount of time. Additionally, no eukaryote plant or animal cell is more primitive with regard to the amount of time they have been subjected to evolutionary mechanisms. Seemingly primitive characteristics are simply highly efficient and conserved characteristics that have changed little over time.

Evolution requires genetic variation, and these variations or changes (**mutations**) can be beneficial, neutral or deleterious. In general, there are two major types of evolutionary mechanisms, those that act to increase genetic variation, and mechanisms that operate to decrease genetic mechanisms.

Mechanisms that increase genetic variation include mutation, **recombination** and gene flow.

Mutations generally occur via chromosomal mutations, point mutations, frame shifts, and breakdowns in **DNA** repair mechanisms. Chromosomal mutations include translocations, inversions, deletions, and chromosome non-disjunction. Point mutations may be nonsense mutations leading to the early termination of **protein synthesis**, missense mutations (a that results an a substitution of one amino acid for another in a protein), or silent mutations that cause no detectable change.

Recombination involves the re-assortment of genes through new chromosome combinations. Recombination occurs via an exchange of DNA between homologous **chromosomes** (crossing over) during meiosis. Recombination also includes linkage disequilibrium. With linkage disequilibrium, variations of the same gene (alleles) occur in combinations in the gametes (sexual reproductive cells) than should occur according to the rules of probability.

Gene flow occurs when gene carriers (e.g., people, bacteria, **viruses**) change their local genetic group by moving—or being transported—from one place to another. These migrations allow the introduction of new variations of the same gene (alleles) when they mate and produce offspring with members of their new group. In effect, gene flow acts to increase the gene pool in the new group. Because genes are usually carried by many members of a large population that has undergone random mating for several generations, random migrations of individuals away from the population or group usually do not significantly decrease the gene pool of the group left behind.

In contrast to mechanisms that operate to increase genetic variation, there are fewer mechanisms that operate to decrease genetic variation. Mechanisms that decrease genetic variation include genetic drift and natural **selection**.

Genetic drift, important to studies of Immunological differences between population groups, results form the

changes in the numbers of different forms of a gene (allelic frequency) that result from sexual reproduction. Genetic drift can occur as a result of random mating (random genetic drift) or be profoundly affected by geographical barriers, catastrophic events (e.g., natural disasters or wars that significantly affect the reproductive availability of selected members of a population), and other political-social factors.

Natural selection is based upon the differences in the viability and reproductive success of different genotypes with a population (differential reproductive success). Natural selection can only act on those differences in **genotype** that appear as phenotypic differences that affect the ability to attract a mate and produce viable offspring that are, in turn, able to live, mate and continue the species. Evolutionary fitness is the success of an entity in reproducing (i.e., contributing alleles to the next generation).

There are three basic types of natural selection. With directional selection, an extreme **phenotype** is favored (e.g., for height or length of neck in giraffe). Stabilizing selection occurs when intermediate **phenotype** is fittest (e.g., neither too high or low a body weight) and for this reason it is often referred to a normalizing selection. Disruptive selection occurs when two extreme phenotypes are fitter that an intermediate phenotype.

Natural selection does not act with foresight. Rapidly changing environmental conditions can, and often do, impose new challenges for a species that result in extinction. In addition, evolutionary mechanisms, including natural selection, do not always act to favor the fittest in any population, but instead may act to favor the more numerous but tolerably fit.

The operation of natural evolutionary mechanisms exhibited in microorganisms is complicated in humans by geographic, ethnic, religious, and social groups and customs. Accordingly, the effects of various evolution mechanisms on human populations are not as easy to predict. Increasingly sophisticated statistical studies are carried out by population geneticists to characterize changes in the human genome, especially with regard to immunological differences between populations.

See also Antibiotic resistance, tests for; Evolutionary origin of bacteria and viruses; Extraterrestrial microbiology; Immunogenetics; Miller-Urey experiment; Molecular biology and molecular genetics; Molecular biology, central dogma of; Mutants, enhanced tolerance or sensitivity to temperature and pH ranges; Mutations and mutagenesis; Radiation mutagenesis; Radiation resistant bacteria; Rare genotype advantage; Viral genetics

Evolutionary origin of bacteria and viruses

Earth formed between 4.5 and 6 billion years ago. Conditions initially remained inhospitable for the potential development of life. By about 3.0 billion years ago, however, an atmosphere that contained the appropriate blend of nitrogen, oxygen, carbon, and hydrogen allowed life to commence. The formation of proteins and nucleic acids led to the generation of the **genetic code**, contained in deoxyribonucleic and ribonucleic acids, and the protein machinery to translate the information into a tangible product.

Fossil evidence indicates that one of the first life forms to arise were **bacteria**. The planetary conditions that were the norm four to six billion years ago were much different from now. Oxygen was scarce, and extremes of factors such as temperature and atmospheric radiation were more common than now. Although the exact origin of bacteria will likely never be known, the present-day bacteria that variously tolerate extremes of temperature, salt concentration, radiation, **pH** and other such environmental factors may be examples of the original bacteria.

Such "**extremophiles**" are part of the division of life known as the Archae, specifically the archaebacteria.

Whether bacteria originated in the sea or on land remains a mystery. The available evidence, however, supports the origin of bacteria in the sea. With the advent of molecular means of comparing the relatedness of bacteria, it has been shown that most of the bacteria known to exist on land bear some resemblance to one another. But, only some 10% of the bacteria from the ocean are in any way related to their terrestrial counterparts. In support of the origin of bacteria in the ancient seas is the discovery of the vast quantities and variety of **viruses** in seawater.

The discovery in the 1970s of bacteria thriving at **hydrothermal vents** deep beneath the surface of the ocean suggests that bacterial life in the ancient oceans was at least certainly possible. Such bacteria would derive their energy from chemical compounds present in their environment. It is also likely that bacterial life was also developing concurrently in response to another energy source, the sun. Indeed, the evolutionarily ancient cyanobacteria are **photosynthetic microorganisms**, which derive their energy from sunlight.

One type of bacteria that is definitely known to have been among the first to appear on Earth is the cyanobacteria. Fossils of cyanobacteria have been uncovered that date back almost 4 billion years. These bacteria are suited to the low oxygen levels that were present in the planet's atmosphere at that time. The cyanobacteria produced oxygen as a waste gas of their metabolic processes and so helped to create an atmosphere containing a greater amount of oxygen. Other, oxygen-requiring bacteria could then develop, along with other life forms.

In contrast to bacteria, scientists debate if viruses are alive. They are not capable of their own reproduction. Instead, they require the presence of a host in which they can introduce their genetic material. Through the formation of products encoded by the viral genetic material and by the use of aspects of the host's replication machinery, viruses are able to direct the manufacture and assembly of components to produce new virus.

The nature of viral replication requires the prior presence of a host. It remains unclear whether the first virus arose from a prokaryotic host, such as a bacterium, or a eukaryotic host. However, the appearance of prokaryotic life prior to

eukaryotic life argues for the origin of viruses as an evolutionary offshoot of prokaryotes.

Scientists are in general agreement that the first virus was a fragment of **DNA** or **ribonucleic acid** (**RNA**) from a eventual prokaryotic or eukaryotic host. The genetic fragment somehow was incorporated into a eukaryotic and became replicated along with the host's genetic material. Over evolutionary time, different viruses developed, having differing specificities for the various bacteria and eukaryotic cells that were arising.

The evolutionary origin of viruses will likely remain conjectural. No fossilized virus has been detected. Indeed, the minute size of viruses makes any distinction of their structure against the background of the rock virtually impossible. Likewise, bacterial fossilization results in the destruction of internal detail. If a virus were to be present in a fossilizing bacterium, any evidence would be obliterated over time.

Some details as to the evolutionary divergence of viruses from a common ancestor are being realized by the comparison of the sequence of evolutionarily maintained sequences of genetic material. This area of investigation is known as virus molecular systematics.

The comparison of a number of **gene** sequences of viral significance, for example the enzyme reverse transcriptase that is possessed by **retroviruses** and pararetroviruses, is consistent with the evolutionary emergence of not one specific type of virus, but rather of several different **types of viruses**. The present day plethora of viruses subsequently evolved from these initial few viral types (or "supergroups" as they have been dubbed). So, in contrast to an evolutionary "tree." viral evolutionary origin resembles more of a bush. Each of the several branches of the bush developed independently of one another. Furthermore, the consensus among virologists (scientists who study viruses) is that this independent **evolution** did not occur at the same time or progress at the same rate. In scientific terms, the viral evolution is described as being "polyphyletic."

The evolution of viruses with life forms, including bacteria, likely occurred together. On other words, as bacteria increased in diversity and in the complexity of their surfaces, new viruses evolved to be able to utilize the bacteria as a replication factory. Similarly, as more complex eukaryotic life forms appeared, such as plants, insects, birds, and mammals, viruses evolved that were capable of utilizing these as hosts.

See also Bacterial kingdoms; Evolution and evolutionary mechanisms; Mitochondrial DNA

EXOTOXIN • *see* ENTEROTOXIN AND EXOTOXIN

EXTRATERRESTRIAL MICROBIOLOGY

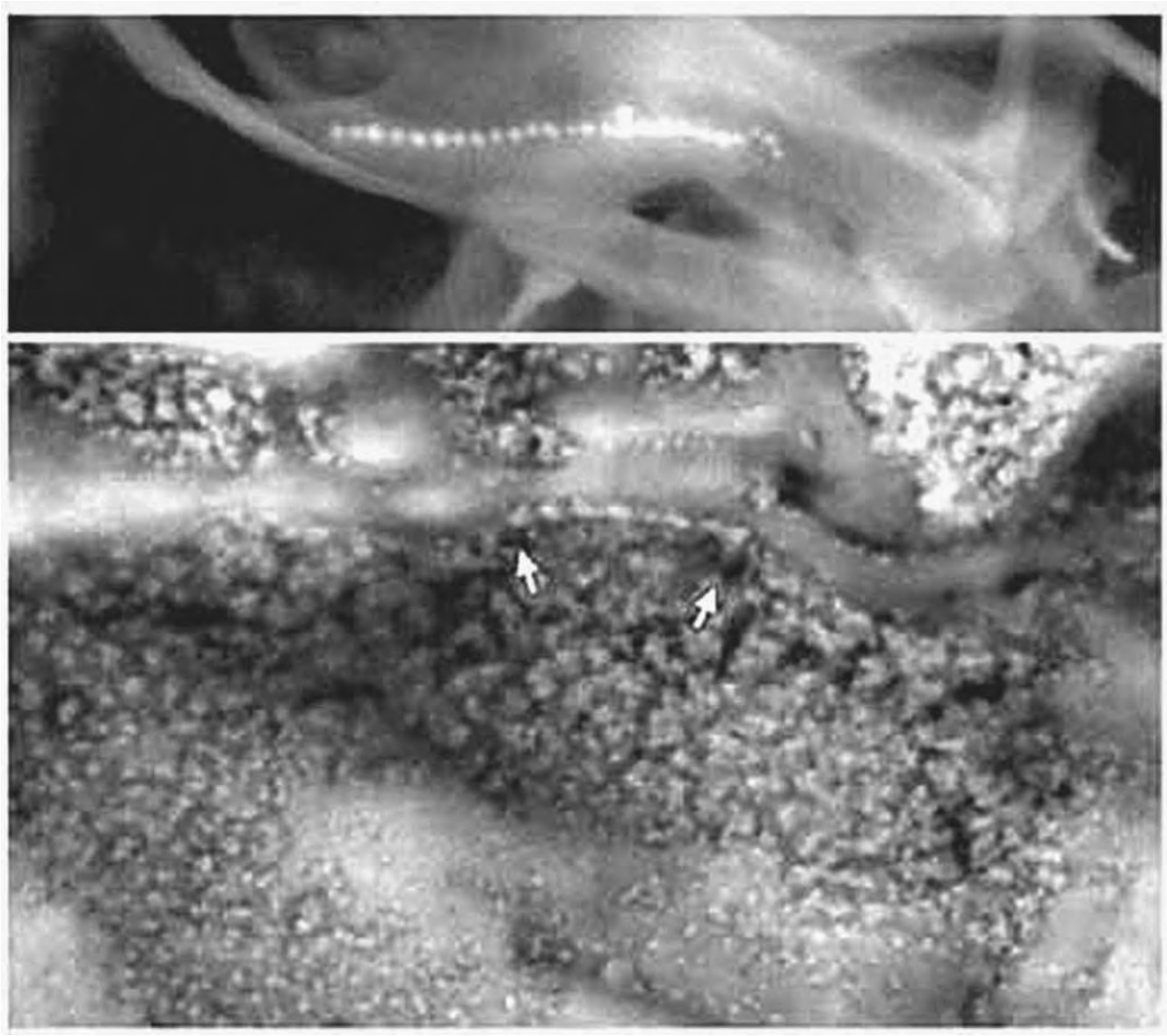
Extraterrestrial microbiology is the study of microbiological processes that could occur outside of the boundaries of Earth, or on other bodies in the solar system. While such **microorganisms** have not yet been found, recent findings of living **bacteria** in very inhospitable environments on Earth, combined with the existence of water on planets such as Mars, have buttressed the possibility that life in similar conditions on other planets is not inconceivable.

The scientific search for extraterrestrial life began in 1860, when the microbiologist **Louis Pasteur** attempted and failed to **culture** bacteria from the Orgueil meteorite.

The search for extraterrestrial life has always been one of the curiosities that has pulled man into the exploration of space. As the chemistries of the planets in our solar system became clearer, the possibilities for human-like life faded. However, at about the same time, the diversity of microbial life on Earth became more apparent. In particular, a type of evolutionarily ancient microorganism known as archaebacteria was isolated from extremely harsh environments, such as hot springs, thermal hot vents on the ocean floor, and from deep in the subsurface of the planet. In contrast to life forms that require oxygen and organic carbon, archaebacteria live on hydrogen and carbon dioxide. Planetary bodies such as Mars and Europa contain atmospheres of hydrogen and carbon dioxide. Thus, theoretically, archaebacteria could find such planets hospitable. Moreover, the finding of bacterial life below the Earth's surface makes the probability of similar life elsewhere greater. Other worlds are more likely to have, or have had, hot and oxygen-limited conditions similar to thermal vents or the subsurface, rather than the sunlight, oxygen-rich atmosphere of Earth's surface. Furthermore, the now prevailing view that archaebacteria are very ancient indicates that life on Earth may have arisen from environments now considered inhospitable. The environment on other solar bodies may be similar to what Earth experienced when microbial life first arose.

Unmanned probes have explored a variety of bodies in our solar system. One such stellar body, Europa, has so far not proved to be a source of life. Probes sent to scan the planet's surface found only lifeless slush. However, two of the moons, which orbit the gas planet Saturn, are of interest. Enceladus has visual signs and chemical signals consistent with the presence of liquid water. The other moon, Titan, is icy and spectral monitoring of the surface has detected signals indicative of organic compounds.

By far the bulk of interest in extraterrestrial microbiology has centered on the planet Mars. Interest in Mars as a potential supporter of microbiological life prompted the *Viking* mission that occurred in 1976. The, in two separate missions, proves landed on different regions of the planet ad conducted experiments designed to detect signature molecules of microbiological activity. One experiment, the gas exchange experiment, sought to detect alterations in the composition of gases in a test chamber. The alterations would presumable be due to microbial decomposition of nutrients, with the consumption of some gases and the release of others. The results were equivocal at first, but with examination were thought to be the result of abiological activity, specifically solar ultraviolet radiation. In a second experiment, radioactive nutrient was released into wetted Martian soil. Bacterial **metabolism** would be evident by the appearance of different radioactive com-



Electron microscopic view of Martian meteorite showing bacteria-like forms.

pounds. Again the results were equivocal, and may have indicated microbiological activity. A third experiment that looked for the presence of organic compounds in the soil was negative. In the final experiment, soil was examined using an instrument called a gas chromatograph-mass spectrometer for chemical signatures of biological activity. The test revealed a great deal of water but little else.

These results have been the subject of debate, and have not proven to be conclusive for the absence of microbiological life. For example, at the time of the *Viking* missions, the full extent of the diversity of microbiological life on Earth, specifically the existence of living bacteria far below the surface in regions where organic material was virtually absent, was not known. With the discovery of archaebacteria, the possibility that microbiological life could exist in the subsurface layers of a planet like Mars has warranted a reassessment of the possibility of Martian life. Furthermore, high resolution photographic surveys of the planet by orbiting probes in the 1990s revealed geological features that are the same as dried rive valleys and floodplains on the Earth. These observations have bolstered the view that Mars was once an abundantly moist planet, capable of sustaining microbiological life.

To definitively address the issue of microbiological life on Mars, the European Space Agency is scheduled to launch the so-called Mars Express in June 2003. The mission will have a two-fold purpose. An orbiting satellite will analyze the planet from high altitude, while a surface probe will sample the planet's surface. The analytical equipment aboard the

probe is designed to detect minute amounts of carbon, and all metabolic forms of the atom. For example, experiments will look for the presence of methane, such as would be produced by methanogenic bacteria.

In addition to extraterrestrial microbial life, interest has arisen over the possibility that extraterrestrial microorganisms could find their way to Earth. Transport of microorganisms via meteorites and on material ejected from the solar body by a meteorite impact has been proposed. In the 1990s, the **electron microscopic examination** of meteorite ALH84001, which originated from Mars, found bacteria-like objects. Their shape, size and chemistry were at the time consistent with a biological origin. However, further study negated this possibility and no other such observations have been made.

See also Anaerobes and anaerobic infections; Biogeochemical cycles; Extremophiles

EXTREMOPHILES

Extremophiles is a term that refers to **bacteria** that are able to exist and thrive in environments that are extremely harsh, in terms of those environments classically envisioned as hospitable to the growth of bacteria.

The discovery of extremophiles, beginning in the 1970s, has had three major influences on microbiology and the **biotechnology** industry. Firstly, the discovery of bacteria growing in environments such as the hot springs of Yellowstone National Park and around the **hydrothermal vents** located on the ocean floor (where the bacteria are in fact the fundamental basis of the specialized ecosystem that is fueled by the vents) has greatly increased the awareness of the possibilities for bacterial life on Earth and elsewhere. Indeed, the growth of some extremophiles occurs in environments that by all indications could exist on planets such as Mars and other stellar bodies. Thus, extremophilic bacteria might conceivably not be confined to Earth.

The second major influence of extremophiles has been the broadening of the classification of the evolutionary development of life on Earth. With the advent of molecular means of comparing the genetic sequences of highly conserved regions from various life forms, it became clear that extremophiles were not simply offshoots of bacteria, but rather had diverged from both bacteria and eukaryotic cells early in evolutionary history. Extremophilic bacteria are grouped together in a domain called **archaea**. Archae share similarities with bacteria and with **eukaryotes**.

Thirdly, extremophiles are continuing to prove to be a rich trove of **enzymes** that are useful in biotechnological processes. The hardiness of the enzymes, such as their ability to maintain function at high temperatures, has been crucial to the development of biotechnology. A particularly well-known example is the so-called tag polymerase enzyme isolated from the extremophile *Thermus aquaticus*. This enzyme is fundamental to the procedures of the **polymerase chain reaction** (**PCR**) procedure that has revolutionized biotechnology.

There are several environments that are inhospitable to all but those extremophilic bacteria that have adapted to live in them. The best studied is elevated temperature. Heat-loving bacteria are referred to as thermophiles. More than 50 species of thermophiles have been discovered to date. Such bacteria tolerate temperatures far above the tolerable limits known for any animal, plant, or other bacteria. Some thermophiles, such as *Sulfolobus acidocaldarius*, are capable of growth and reproduction in water temperatures that exceed 212° F [100° C] (the boiling point of water at sea level). The most heat-tolerant thermophile known so far is *Pyrolobus fumarii*, that grows in the walls of the hydrothermal vents where temperatures exceed 200° F [93.33° C]. In fact, the bacterium requires a temperature above 194° F [90° C] to sustain growth. The basis of the thermophile's ability to prevent dissolution of cell wall constituents and genetic material at such high temperatures is unknown.

Other examples of extreme environments include elevated salt, pressure, and extreme acid or base concentrations.

Salt-loving, or halophilic, bacteria grow in environments where the sodium concentration is extremely high, such as in the Dead Sea or Great Salt Lake. In such an environment, a bacterium such as ***Escherichia coli*** would compensate for the discrepancy in sodium concentration between the bacterium's interior and exterior by shunting all the internal fluid to the exterior. The result would be the collapse and death of the bacterium. However, salt-loving bacteria such as *Halobacterium salinarum* content with the sodium discrepancy by increasing the internal concentration of potassium chloride. The enzymes of the bacterium operate only in a potassium chloride-rich environment. Yet the proteins produced by the action of these enzymes need to be tolerant of high sodium chloride levels. How the enzymes are able to accommodate both demands is not clear.

Acid-loving extremophiles prefer environments where the **pH** is below pH=5, while alkaline-loving bacteria require pHs above pH=9. Thriving populations of acid-loving bacteria have been isolated in the runoff from acidic mine drainage, where the pH is below one, which is more acidic than the contents of the stomach. Interestingly, these bacteria are similar to other bacteria in the near neutral pH of their interior. Very acidic pHs would irreversibly damage the genetic material. Acid-loving bacteria thus survive by actively excluding acid. The enzymes necessary to achieve this function at very acidic pH levels.

Similarly, alkaline-loving bacteria maintain a near neutral interior pH. The enzymes that function at such alkaline conditions are of interest to manufacturers of laundry detergents, which operate better at alkaline pHs.

Some extremophiles grow and thrive at very low temperatures. For example, *Polaromonas vacuolata* has an ideal growth temperature of just slightly above the freezing point of water. These bacteria are finding commercial applications in enzymatic processes that operate at refrigeration temperatures or in the cold cycle of a washing machine.

The discovery of bacteria in environments that were previously disregarded as being completely inhospitable for bacterial life argues that more extremophiles are yet to be

A steaming hot spring in Yellowstone National Park.

found, as other environments are explored. For example, in 2001, living bacteria were recovered from drill samples kilometers beneath the Earth's crust, in an environment where virtually no nutrients were present other than the solid rock surrounding the bacteria. By as yet unknown enzymatic mechanisms, these bacteria are able to extract elemental components including sulfur from the rocks and utilize them as nutrients.

See also Chemoautotrophic and chemolithotrophic bacteria; Economic uses and benefits of microorganisms; Extraterrestrial microbiology

EYE INFECTIONS

Eye infections can be caused by viral, bacterial, and fungal **microorganisms**. These organisms do not cause infections solely in the eye. In reality, eye infections tend to occur as infections disseminate, or spread, in the body.

Microbiological infections of the eye involve the conjunctiva, which is the membrane of the inner eyelid and corner of the eye. This infection is termed conjunctivitis. Depending on the microbial agent, infection can also occur on the eyelid (blepharitis), the cornea (keratitis), the retina and its associated blood vessels (chorioretinitis), the optic nerve (neuroritinitis), and even the fluid inside the eyeball (vitritis).

A virus associated with eye infections is the **Herpes** Zoster virus. This virus is the reactivated form of the chicken pox virus that had previously established an infection, often in childhood. A hallmark of reactivation is the dissemination of the virus throughout the body via nerve fibers. The eye can become infected through the optic nerve fibers.

Typically, the viral infection will be a rash or **inflammation** on the upper or lower eyelid and the conjunctiva. The inside of the eye and the optic nerve leading from the retina to

the brain can also become infected. Herpes Zoster eye infections can produce redness, swelling, pain, light sensitivity, and blurred vision.

The cornea of the eye is prone to infection by the type of **fungi** known as molds, and by **yeast**. Such an infection is termed mycotic keratitis. Infections can arise following eye surgery, from the use of contaminated contact lens (or the **contamination** of the contact lens cleaning solution), or due to a malfunction of the **immune system**. A common fungal cause of eye infections are species of *Aspergillus*. A common yeast source of infection are species of *Candida*. The eye infection may be a secondary result of the spread of a fungal or yeast infection elsewhere in the body. For example, those afflicted with acquired **immunodeficiency** syndrome can develop eye infections in addition to other fungal or yeast maladies.

Bacterial eye infections are often caused by *Chlamydia*, *Neiserria*, and *Pseudomonas*. The latter **bacteria**, which can infect the fluid used to clean contact lenses, can cause the rapid development of an infection that can so severe that blindness can result. Removal of the infected eye is sometimes necessary to stop the infection.

Less drastic solutions to infections include the use of antimicrobial eye drops.

See also Immune system

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FACILITATED DIFFUSION • *see* CELL MEMBRANE TRANSPORT

FAUCI, ANTHONY S. (1940-)

American immunologist

Early in his career, Anthony S. Fauci carried out both basic and clinical research in **immunology** and infectious diseases. Since 1981, Fauci's research has been focused on the mechanisms of the **Human Immunodeficiency Virus** (**HIV**), which causes acquired **immunodeficiency** syndrome (**AIDS**). His work has lead to breakthroughs in understanding the virus's progress, especially during the latency period between infection and fulminant AIDS. As director of both the National Institute of Allergy and Infectious Diseases (NIAID) and the Office of AIDS Research at the National Institutes of Health (NIH), Fauci is involved with much of the AIDS research performed in the United States and is responsible for supervising the investigation of the disease mechanism and the development of vaccines and drug therapy.

Anthony Stephen Fauci was born on December 24, 1940, in Brooklyn, New York, to Stephen A. Fauci, a pharmacist, and Eugenia A. Fauci, a homemaker. He attended a Jesuit high school in Manhattan where he had a successful academic and athletic career. After high school, Fauci entered Holy Cross College in Worcester, Massachusetts, as a premedical student, graduating with a B.A. in 1962. He then attended Cornell University Medical School, from which he received his medical degree in 1966, and where he completed both his internship and residency.

In 1968, Fauci became a clinical associate in the Laboratory of Clinical Investigation of NIAID, one of the eleven institutes that comprise the NIH. Except for one year spent at the New York Hospital Cornell Medical Center as chief resident, he has remained at the NIH throughout his career. His earliest studies focused on the functioning of the human **immune system** and how infectious diseases impact the system. As a senior staff fellow at NIAID, Fauci and two other researchers delineated the mechanism of Wegener's granulomatosis, a relatively rare and fatal immune disease involving the **inflammation** of blood vessels and organs. By 1971, Fauci had developed a drug regimen for Wegener's granulomatosis that is 95% percent effective. He also found effective treatments for lymphomatoid granulomatosis and polyarteritis nodosa, two other immune diseases.

In 1972, Fauci became a senior investigator at NIAID and two years later he was named head of the Clinical Physiology Section. In 1977, Fauci was appointed deputy clinical director of NIAID. Fauci shifted the focus of the Laboratory of Clinical Infection at NIAID towards investigating the nature of AIDS in the early 1980s. It was in Fauci's lab the type of defect that occurs in the T4 helper cells (the immune cells) and enables AIDS to be fatal was demonstrated. Fauci also orchestrated early therapeutic techniques, including bone-marrow transplants, in an attempt to save AIDS patients. In 1984, Fauci became the director of NIAID, and the following year the coordinator of all AIDS research at NIH. He has worked not only against the disease but also against governmental indifference to AIDS, winning larger and larger budgets for AIDS research. When the Office of AIDS Research at NIH was founded in 1988, Fauci was made director; he also decided to remain the director of NIAID. Fauci and his research teams have developed a three-fold battle plan against AIDS: researching the mechanism of HIV, developing and testing drug therapies, and creating an AIDS **vaccine**.

In 1993, Fauci and his team at NIH disproved the theory that HIV remains dormant for approximately ten years after the initial infection, showing instead that the virus attacks the lymph nodes and reproduces itself in white blood cells known as CD4 cells. This discovery could lead to new and radical approaches in the early treatment of HIV-positive patients. Earlier discoveries that Fauci and his lab are responsible for include the 1987 finding that a protein substance known as cytokine may be responsible for triggering full-blown AIDS

and the realization that the macrophage, a type of immune system cell, is the virus's means of transmission. Fauci demonstrated that HIV actually hides from the body's immune system in these macrophages and is thus more easily transmitted. In an interview with Dennis L. Breo published in the *Journal of the American Medical Association,* Fauci summed up his research to date: "We've learned that AIDS is a multiphasic, multifactorial disease of overlapping phases, progressing from infection to viral replication to chronic smoldering disease to profound depression of the immune system."

In drug therapy work, Fauci and his laboratory have run hundreds of clinical tests on medications such as azidothymidine (AZT), and Fauci has pushed for the early use of such drugs by terminally ill AIDS patients. Though no completely effective antiviral drug yet exists, drug therapies have been developed that can prolong the life of AIDS victims. Potential AIDS vaccines are still being investigated, a process complicated by the difficulty of conducting possible clinical trials, and the fact that animals do not develop AIDS as humans do, which further limits available research subjects. No viable vaccine is expected before the year 2005.

As chief government infectious disease specialist, Fauci was presented with an immediate **public health** challenge in October, 2001—bioterrorism. Coordinating with the **Centers for Disease Control**, Fauci directed the effort to not only contain the outbreak of **anthrax** resulting from *Bacillus anthracis*–contaminated letters mailed to United States Post Offices, but also to initiate the necessary research to manage the continuing threat of the disease. Fauci also labeled **smallpox** as a logical **bioterrorism** agent, and has concentrated his efforts to ensure an available adequate supply of smallpox vaccine in the U.S.

Fauci married Christine Grady, a clinical nurse and medical ethicist, in 1985. The couple has three daughters. Fauci is an avid jogger, a former marathon runner, and enjoys fishing. Widely recognized for his research, he is the recipient of numerous prizes and awards, including a 1979 Arthur S. Flemming Award, the 1984 U.S. Public Health Service Distinguished Service Medal, the 1989 National Medical Research Award from the National Health Council, and the 1992 Dr. Nathan Davis Award for Outstanding Public Service from the American Medical Association. Fauci is also a fellow of the American Academy of Arts and Sciences and holds a number of honorary degrees. He is the author or coauthor of over 800 scientific articles, and has edited several medical textbooks.

See also AIDS, recent advances in research and treatment; Anthrax, terrorist use of as a biological weapon; Bioterrorism, protective measures; Epidemiology, tracking diseases with technology; Infection and resistance

FELDMAN, HARRY ALFRED (1914-1985)

American physician and epidemiologist

Harry A. Feldman's research in **epidemiology**, **immunology**, infectious disease control, preventive medicine, **toxoplasmosis**, bacterial chemotherapeutic and sero-therapeutic agents, respiratory diseases, and **meningitis** was internationally recognized in the scientific community of microbiology and medicine.

Feldman was born in Newark, New Jersey on May, 30, 1914, the son of Joseph Feldman, a construction contractor, and his wife Sarah. After attending public schools in Newark and graduating from Weequahic High School in 1931, he received his A.B. in zoology in 1935 and his M.D. in 1939, both from George Washington University. He completed an internship and residency at Gallinger Municipal Hospital, Washington, D.C., held a brief research fellowship at George Washington, then in 1942, became a research fellow at Harvard Medical School and an assistant resident physician at the Boston City Hospital's Thorndike Memorial Laboratory. Among his colleagues at Thorndike was Maxwell A. Finland (1902–1987), who at the time was among the nation's premier investigators of infectious diseases. From 1942 to 1946, Feldman served to the rank of lieutenant colonel in the United States Army Medical Corps.

As senior fellow in virus diseases for the National Research Council at the Children's Hospital Research Foundation, Cincinnati, Ohio, Feldman collaborated with Albert B. Sabin (1906–1993) on **poliomyelitis** and toxoplasmosis from 1946 to 1948. Together they developed the Sabin-Feldman dye test, which uses methylene blue to detect toxoplasmosis in blood serum by identifying immunoglobulin-G (IgG) antibodies against the parasitic intracellular protozoan, *toxoplasma gondii*.

In 1948, Feldman was appointed associate professor of medicine at the Syracuse University College of Medicine, which in 1950 became the State University of New York Upstate Medical Center College of Medicine. From 1949 to 1956, he also served in Syracuse as director of research at the Wieting-Johnson Hospital for Rheumatic Diseases. In 1955, Upstate named him associate professor of preventive medicine. The following year he was promoted to full professor and in 1957, became chair of the Department of Preventive Medicine, the position he held until his death. Between 1938 and 1983, he published 216 research papers, both in scientific journals and as book chapters. With Alfred S. Evans (1917-1996), he co-edited *Bacterial Infections of Humans* (1982).

Besides his groundbreaking work on toxoplasmosis, both with Sabin in Cincinnati and later as head of his own team in Syracuse, Feldman regarded his work on meningococcus and on parasitic **protozoa** such as acanthamoeba as his greatest contributions to science. Among the diseases he studied were **malaria**, **pneumonia**, rubella, **measles**, **influenza**, streptococcal infections, and **AIDS**. He conducted extensive clinical pharmaceutical trials and served enthusiastically as a member of many scientific organizations, commissions, and committees, including the **World Health Organization** (**WHO**) expert advisory panels on bacterial diseases, venereal diseases, treponematoses, and neisseria infections. He was president of the American Epidemiological Society (AES), the Infectious Diseases Society of America (IDSA), and the Association of Teachers of Preventive Medicine. The AES established the Harry A. Feldman Lectureship and the Harry

A. Feldman Award in his honor, and the IDSA also created its own Harry A. Feldman Award.

See also Antibody and antigen; Bacteria and bacterial infection; Chemotherapy; Epidemiology; Infection and resistance; Meningitis, bacterial and viral; Microbiology, clinical; Parasites; Poliomyelitis and polio; Protozoa; Serology

FERMENTATION

In its broadest sense, fermentation refers to any process by which large organic molecules are broken down to simpler molecules as the result of the action of **microorganisms**. The most familiar type of fermentation is the conversion of sugars and starches to alcohol by **enzymes** in **yeast**. To distinguish this reaction from other kinds of fermentation, the process is sometimes known as alcoholic or ethanolic fermentation.

Ethanolic fermentation was one of the first chemical reactions observed by humans. In nature, various types of spoil decompose because of bacterial action. Early in history, humans discovered that this kind of change could result in the formation of products that were enjoyable to consume. The spoilage (fermentation) of fruit juices, for example, resulted in the formation of primitive forms of wine.

The mechanism by which fermentation occurs was the subject of extensive debate in the early 1800s. It was a key issue among those arguing over the concept of vitalism, the notion that living organisms are in some way inherently different from non-living objects. One aspect in this debate centered on the role of so-called "ferments" in the conversion of sugars and starches to alcohol. Vitalists argued that ferments (now known as enzymes) are inextricably linked to a living cell; destroy a cell and ferments can no longer cause fermentation, they argued.

A crucial experiment on this issue was carried out in 1896 by the German chemist Eduard Buchner. Buchner ground up a group of cells with sand until they were totally destroyed. He then extracted the liquid that remained and added it to a sugar solution. His assumption was that fermentation could no longer occur because the cells that had held the ferments were dead, so they no longer carried the "life-force" needed to bring about fermentation. He was amazed to discover that the cell-free liquid did indeed cause fermentation. It was obvious that the ferments themselves, distinct from any living organism, could cause fermentation.

The chemical reaction that occurs in fermentation can be described easily. Starch is converted to simple sugars such as sucrose and glucose. Those sugars are then converted to alcohol (ethyl alcohol) and carbon dioxide. This description does not adequately convey the complexity of the fermentation process itself. During the 1930s, two German biochemists, G. Embden and O. Meyerhof, worked out the sequence of reactions by which glucose ferments. In a sequence of twelve reactions, glucose is converted to ethyl alcohol and carbon dioxide. A number of enzymes are needed to carry out this sequence of reactions, the most important of which is zymase, found in yeast cells. These enzymes are sensitive to environmental conditions in which they live. When the concentration of alcohol reaches about 14%, they are inactivated. For this reason, no fermentation product (such as wine) can have an alcoholic concentration of more than about fourteen percent.

Large vats in which the fermentation process in the brewing of beer occurs.

The alcoholic beverages that can be produced by fermentation vary widely, depending primarily on two factors—the plant that is fermented and the enzymes used for fermentation. Human societies use, of course, the materials that are available to them. Thus, various peoples have used grapes, berries, corn, rice, wheat, honey, potatoes, barley, hops, cactus juice, cassava roots, and other plant materials for fermentation. The products of such reactions are various forms of beer, wine or distilled liquors, which may be given specific names depending on the source from which they come. In Japan, for example, rice wine is known as sake. Wine prepared from honey is known as mead. Beer is the fermentation product of barley, hops, and/or malt sugar.

Early in human history, people used naturally occurring yeast for fermentation. The products of such reactions

depended on whatever enzymes might occur in "wild" yeast. Today, wine-makers are able to select from a variety of specially cultured yeast that control the precise direction that fermentation will take.

Ethyl alcohol is not the only useful product of fermentation. The carbon dioxide generated during fermentation is also an important component of many baked goods. When the batter for bread is mixed, for example, a small amount of sugar and yeast is added. During the rising period, sugar is fermented by enzymes in the yeast, with the formation of carbon dioxide gas. The carbon dioxide gives the batter bulkiness and texture that would be lacking without the fermentation process. Fermentation has a number of commercial applications beyond those described thus far. Many occur in the food preparation and processing industry. A variety of **bacteria** are used in the production of olives, cucumber pickles, and sauerkraut from the raw olives, cucumbers, and cabbage, respectively. The **selection** of exactly the right bacteria and the right conditions (for example, acidity and salt concentration) is an art in producing food products with exactly the desired flavors. An interesting line of research in the food sciences is aimed at the production of edible food products by the fermentation of petroleum.

In some cases, **antibiotics** and other drugs can be prepared by fermentation if no other commercially efficient method is available. For example, the important drug cortisone can be prepared by the fermentation of a plant steroid known as diosgenin. The enzymes used in the reaction are provided by the **mold** *Rhizopus nigricans*.

One of the most successful commercial applications of fermentation has been the production of ethyl alcohol for use in gasohol. Gasohol is a mixture of about 90% gasoline and 10% alcohol. The alcohol needed for this product can be obtained from the fermentation of agricultural and municipal wastes. The use of gasohol provides a promising method for using renewable resources (plant material) to extend the availability of a nonrenewable resource (gasoline).

Another application of the fermentation process is in the treatment of wastewater. In the activated sludge process, aerobic bacteria are used to ferment organic material in wastewater. Solid wastes are converted to carbon dioxide, water, and mineral salts.

See also History of microbiology; Winemaking

FERTILITY • *see* REPRODUCTIVE IMMUNOLOGY

FILOVIRUSES • *see* HEMORRHAGIC FEVERS AND DISEASES

FIMBRIA • *see* BACTERIAL APPENDAGES

FLAGELLA • *see* BACTERIAL APPENDAGES

FLAVIVIRUSES • *see* HEMORRHAGIC FEVERS AND DISEASES

FLEMING, ALEXANDER (1881-1955)

Scottish bacteriologist

With the experienced eye of a scientist, Alexander Fleming turned what appeared to be a spoiled experiment into the discovery of **penicillin**.

Fleming was born in 1881 to a farming family in Lochfield, Scotland. Following school, he worked as a shipping clerk in London and enlisted in the London Scottish Regiment. In 1901, he began his medical career, entering St. Mary's Hospital Medical School, where he was a prizewinning student. After graduation in 1906, he began working at that institution with Sir **Almroth Edward Wright**, a pathologist. From the start, Fleming was innovative and became one of the first to use **Paul Ehrlich**'s arsenic compound, Salvarsan, to treat **syphilis** in Great Britain.

Wright and Fleming joined the Royal Army Medical Corps during World War I and they studied wounds and infection-causing **bacteria** at a hospital in Boulogne, France. At that time, **antiseptics** were used to treat bacterial infections, but Wright and Fleming showed that, especially in deep wounds, bacteria survive treatment by antiseptics while the protective white blood cells in the wound are destroyed. This creates an even worse situation in which infection can spread rapidly. Forever affected by the suffering he saw during the war, Fleming decided to focus his efforts on the search for safe antibacterial substances. He studied the antibacterial power of the body's own leukocytes contained in pus. In 1921, he discovered that a sample of his own nasal mucus destroyed bacteria in a petri dish. He isolated the compound responsible for the antibacterial action, which he called lysozyme, in saliva, blood, tears, pus, milk, and in egg whites.

Fleming made his greatest discovery in 1928. While he was growing cultures of bacteria in petri dishes for experiments, he accidentally left certain dishes uncovered for several days. Fleming found a **mold** growing in the dishes and began to discard them, when he noticed, to his astonishment, that bacteria near the molds were being destroyed. He preserved the mold, a strain of Penicillium and made a **culture** of it in a test tube for further investigation. He deduced an antibacterial compound was being produced by the mold, and named it penicillin. Through further study, Fleming found that penicillin was nontoxic in laboratory animals. He described his findings in research journals but was unable to purify and concentrate the substance. Little did he realize that the substance produced by his mold would save millions of lives during the twentieth century.

Fleming dropped his investigation of penicillin and his discovery remained unnoticed until 1940. It was then that Oxford University-based bacteriologists Howard Florey and Ernst Chain stumbled upon a paper by Fleming while researching antibacterial agents. They had better fortune than Fleming, for they were able to purify penicillin and test it on humans with outstanding results. During World War II, the drug was rushed into mass-production in England and the United States and saved thousands of injured soldiers from infections that might otherwise have been fatal.

Sir Alexander Flemming, the discoverer of lysozyme and penicillin.

Accolades followed for Fleming. He was elected to fellowship in the Royal Society in 1943, knighted in 1944, and shared the Nobel Prize with Florey and Chain in 1945. Fleming continued working at St. Mary's Hospital until 1948, when he moved to the Wright-Fleming Institute. Fleming died in London in 1955.

See also Antibiotic resistance, tests for; Antibiotics; Bacteria and bacterial infection; History of the development of antibiotics; History of microbiology; History of public health

Florey, Howard Walter (1898-1968)

English pathologist

The work of Howard Walter Florey gave the world one of its most valuable disease-fighting drugs, **penicillin**. **Alexander Fleming** discovered, in 1929, the **mold** that produced an antibacterial substance, but was unable to isolate it. Nearly a decade later, Florey and his colleague, biochemist **Ernst Chain**, set out to isolate the active ingredient in Fleming's mold and then conduct the clinical tests that demonstrated penicillin's remarkable therapeutic value. Florey and Chain reported the initial success of their clinical trials in 1940, and the drug's value was quickly recognized. In 1945, Florey shared the Nobel Prize in medicine or physiology with Fleming and Chain.

Howard Walter Florey was born in Adelaide, Australia. He was one of three children and the only son born to Joseph Florey, a boot manufacturer, and Bertha Mary Wadham Florey, Joseph's second wife. Florey expressed an interest in science early in life. Rather than follow his father's career path, he decided to pursue a degree in medicine. Scholarships afforded him an education at St. Peter's Collegiate School and Adelaide University, the latter of which awarded him a Bachelor of Science degree in 1921. An impressive academic career earned Florey a Rhodes scholarship to Oxford University in England. There he enrolled in Magdalen College in January 1922. His academic prowess continued at Oxford, where he became an excellent student of physiology under the tutelage of renowned neurophysiologist Sir Charles Scott Sherrington. Placing first in his class in the physiology examination, he was appointed to a teaching position by Sherrington in 1923.

Florey's education continued at Cambridge University as a John Lucas Walker Student. Already fortunate enough to have learned under a master such as Sherrington, he now came under the influence of Sir Frederick Gowland Hopkins, who taught Florey the importance of studying biochemical reactions in cells. A Rockefeller Traveling Scholarship sent Florey to the United States in 1925, to work with physiologist Alfred Newton Richards at the University of Pennsylvania, a collaboration that would later prove beneficial to Florey's own research. On his return to England and Cambridge in 1926, Florey received a research fellowship in pathology at London Hospital. That same year, he married Mary Ethel Hayter Reed, an Australian whom he'd met during medical school at Adelaide University. The couple eventually had two children.

Florey received his Ph.D. from Cambridge in 1927, and remained there as Huddersfield Lecturer in Special Pathology. Equipped with a firm background in physiology, he was now in a position to pursue experimental research using an approach new to the field of pathology. Instead of describing diseased tissues and organs, Florey applied physiologic concepts to the study of healthy biological systems as a means of better recognizing the nature of disease. It was during this period in which Florey first became familiar with the work of Alexander Fleming. His own work on mucus secretion led him to investigate the intestine's resistance to **bacterial infection**. As he became more engrossed in antibacterial substances, Florey came across Fleming's report of 1921 describing the enzyme lysozyme, which possessed antibacterial properties. The enzyme, found in the tears, nasal secretions, and saliva of humans, piqued Florey's interest, and convinced him that collaboration with a chemist would benefit his research. His work with lysozyme showed that extracts from natural substances, such as plants, **fungi** and certain types of **bacteria**, had the ability to destroy harmful bacteria.

Florey left Cambridge in 1931 to become professor of pathology at the University of Sheffield, returning to Oxford in 1935 as director of the new Sir William Dunn School of Pathology. There, at the recommendation of Hopkins, his productive collaboration began with the German biochemist Ernst Chain. Florey remained interested in antibacterial substances

even as he expanded his research projects into new areas, such as cancer studies. During the mid 1930s, sulfonamides, or **sulfa drugs**, had been introduced as clinically effective against streptococcal infections, an announcement which boosted Florey's interest in the field. At Florey's suggestion, Chain undertook biochemical studies of lysozyme. He read much of the scientific literature on antibacterial substances, including Fleming's 1929 report on the antibacterial properties of a substance extracted from a Penicillium mold, which he called penicillin. Chain discovered that lysozyme acted against certain bacteria by catalyzing the breakdown of polysaccharides in them, and thought that penicillin might also be an enzyme with the ability to disrupt some bacterial component. Chain and Florey began to study this hypothesis, with Chain concentrating on isolating and characterizing the enzyme, and Florey studying its biological properties.

To his surprise, Chain discovered that penicillin was not a protein, therefore it could not be an enzyme. His challenge now was to determine the chemical nature of penicillin, made all the more difficult because it was so unstable in the laboratory. It was, in part, for this very reason that Fleming eventually abandoned a focused pursuit of the active ingredient in Penicillium mold. Eventually, work by Chain and others led to a protocol for keeping penicillin stable in solution. By the end of 1938, Florey began to seek funds to support more vigorous research into penicillin. He was becoming convinced that this antibacterial substance could have great practical clinical value. Florey was successful in obtaining two major grants, one from the Medical Research Council in England, the other from the Rockefeller Foundation in the United States.

By March of 1940, Chain had finally isolated about one hundred milligrams of penicillin from broth cultures. Employing a freeze-drying technique, he extracted the yellowish-brown powder in a form that was yet only ten percent pure. It was non-toxic when injected into mice and retained antibacterial properties against many different pathogens. In May of 1940, Florey conducted an important experiment to test this promising new drug. He infected eight mice with lethal doses of **streptococci** bacteria, then treated four of them with penicillin. The following day, the four untreated mice were dead, while three of the four mice treated with penicillin had survived. Though one of the mice that had been given a smaller dose died two days later, Florey showed that penicillin had excellent prospects, and began additional tests. In 1941, enough penicillin had been produced to run the first clinical trial on humans. Patients suffering from severe staphylococcal and streptococcal infections recovered at a remarkable rate, bearing out the earlier success of the drugs in animals. At the outset of World War II, however, the facilities needed to produce large quantities of penicillin were not available. Florey went to the United States where, with the help of his former colleague, Alfred Richards, he was able to arrange for a U.S. government lab to begin large-scale penicillin production. By 1943, penicillin was being used to treat infections suffered by wounded soldiers on the battlefront.

Recognition for Florey's work came quickly. In 1942, he was elected a fellow in the prestigious British scientific organization, the Royal Society, even before the importance of penicillin was fully realized. Two years later, Florey was knighted. In 1945, Florey, Chain and Fleming shared the Nobel Prize in medicine or physiology for the discovery of penicillin.

Penicillin prevents bacteria from synthesizing intact cell walls. Without the rigid, protective cell wall, a bacterium usually bursts and dies. Penicillin does not kill resting bacteria, only prevents their proliferation. Penicillin is active against many of the gram positive and a few gram negative bacteria. (The gram negative/positive designation refers to a staining technique used in identification of microbes.) Penicillin has been used in the treatment of **pneumonia**, **meningitis**, many throat and ear infections, Scarlet Fever, endocarditis (heart infection), **gonorrhea**, and **syphilis**.

Following his work with penicillin, Florey retained an interest in antibacterial substances, including the cephalosporins, a group of drugs that produced effects similar to penicillin. He also returned to his study of capillaries, which he had begun under Sherrington, but would now be aided by the recently developed **electron microscope**. Florey remained interested in Australia, as well. In 1944, the prime minister of Australia asked Florey to conduct a review of the country's medical research. During his trip, Florey found laboratories and research facilities to be far below the quality he expected. The trip inspired efforts to establish graduate-level research programs at the Australian National University. For a while, it looked as if Florey might even return to Australia to head a new medical institute at the University. That never occurred, although Florey did do much to help plan the institute and recruit scientists to it. During the late 1940s and 1950s, Florey made trips almost every year to Australia to provide consultation to the new Australian National University, to which he was appointed Chancellor in 1965.

Florey's stature as a scientist earned him many honors in addition to the Nobel Prize. In 1960, he became president of the Royal Society, a position he held until 1965. Tapping his experience as an administrator, Florey invigorated this prestigious scientific organization by boosting its membership and increasing its role in society. In 1962, he was elected Provost of Queen's College, Oxford University, the first scientist to hold that position. He accepted the presidency of the British Family Planning Association in 1965, and used the post to promote more research on contraception and the legalization of abortion. That same year, he was granted a peerage, becoming Baron Florey of Adelaide and Marston.

See also Bacteria and bacterial infection; History of the development of antibiotics; Infection and resistance

Flu: The great flu epidemic of 1918

From 1918 to 1919, an outbreak of **influenza** ravaged Europe and North America. The outbreak was a pandemic; that is, individuals in a vast geographic area were affected. In the case

of this particular influenza outbreak, people were infected around the world.

The pandemic killed more people, some 20 to 40 million, than had been killed in the just-ending Great War (now known as World War I). Indeed, the pandemic is still the most devastating microbiological event in the recorded history of the world. At the height of the epidemic, fully one-fifth of the world's population was infected with the virus.

The disease first arose in the fall of 1918, as World War I was nearing its end. The genesis of the disease caused by the strain of influenza virus may have been the deplorable conditions experienced by soldiers in the trenches that were dug at battlegrounds throughout Europe. The horrible conditions rendered many soldiers weak and immunologically impaired. As solders returned to their home countries, such as the United States, the disease began to spread. As the disease spread, however, even healthy people fell victim to the infection. The reason why so many apparently healthy people would suddenly become ill and even die was unknown at the time. Indeed, the viral cause of disease had yet to be discovered.

Recent research has demonstrated that the particular strain of virus was one that even an efficiently functioning **immune system** was not well equipped to cope with. A mutation produced a surface protein on the virus that was not immediately recognized by the immune system, and which contributed to the ability of the virus to cause an infection.

The influenza outbreak has also been called the "Spanish Flu" or "La Grippe." The moniker came from the some 8 million influenza deaths that occurred in Spain in one month at the height of the outbreak. Ironically, more recent research has demonstrated that the strain of influenza that ravaged Spain was different from that which spread influenza around the world.

The influenza swept across Europe and elsewhere around the globe. In the United States, some 675,000 Americans perished from the infection, which was brought to the continent by returning war veterans. The outbreaks in the United States began in military camps. Unfortunately, the significance of the illness was not recognized by authorities and few steps were taken to curtail the illnesses, which soon spread to the general population.

The resulting carnage in the United States reduced the statistical average life span of an American by 10 years. In the age range of 15 to 34 years, the death rate in 1918 due to **pneumonia** and influenza was 20 times higher than the normal rate. The large number of deaths in many of the young generation had an economic effect for decades to come. South America, Asia, and the South Pacific were also devastated by the infection.

In the United States the influenza outbreak greatly affected daily life. Gatherings of people, such as at funerals, parades, or even sales at commercial establishments were either banned or were of very short duration. The medical system was taxed tremendously.

The influenza outbreak of 1918 was characterized by a high mortality rate. Previous influenza outbreaks had displayed a mortality rate of far less than 1%. However, the 1918 pandemic had a much higher mortality rate of 2.5%. Also, the illness progressed very quickly once the symptoms of infections appeared. In many cases, an individual went from a healthy state to serious illness or death with 24 hours.

At the time of the outbreak, the case of the illness was not known. Speculations as to the source of the illness included an unknown weapon of war unleashed by the German army. Only later was the viral origin of the disease determined. In the 1970s, a study that involved a genetic characterization of viral material recovered from the time of the pandemic indicated that the strain of the influenza virus likely arose in China, and represented a substantial genetic alteration from hitherto known viral types.

In November of 1919, the influenza outbreak began to disappear as rapidly as it had appeared. With the hindsight of present day knowledge of viral epidemics, it is clear that the number of susceptible hosts for the virus became exhausted. The result was the rapid end to the epidemic.

See also Epidemics, viral; History of public health

FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

Fluorescent *in situ* hybridization (FISH) is a technique in which single-stranded nucleic acids (usually **DNA**, but **RNA** may also be used) are permitted to interact so that complexes, or hybrids, are formed by molecules with sufficiently similar, complementary sequences. Through nucleic acid hybridization, the degree of sequence identity can be determined, and specific sequences can be detected and located on a given chromosome. It is a powerful technique for detecting RNA or DNA sequences in cells, tissues, and tumors. FISH provides a unique link among the studies of cell biology, cytogenetics, and **molecular genetics**.

The method is comprised of three basic steps: fixation of a specimen on a **microscope** slide, hybridization of labeled probe to homologous fragments of genomic DNA, and enzymatic detection of the tagged probe-target hybrids. While probe sequences were initially detected with isotopic reagents, nonisotopic hybridization has become increasingly popular, with fluorescent hybridization now a common choice. Protocols involving nonisotopic probes are considerably faster, with greater signal resolution, and provide options to visualize different targets simultaneously by combining various detection methods.

The detection of sequences on the target **chromosomes** is performed indirectly, commonly with biotinylated or digoxigenin-labeled probes detected via a fluorochrome-conjugated detection reagent, such as an **antibody** conjugated with fluorescein. As a result, the direct visualization of the relative position of the probes is possible. Increasingly, nucleic acid probes labeled directly with fluorochromes are used for the detection of large target sequences. This method takes less time and results in lower background; however, lower signal intensity is generated. Higher sensitivity can be obtained by building layers of detection reagents, resulting in amplification of the sig-

nal. Using such means, it is possible to detect single-copy sequences on chromosome with probes shorter than 0.8 kb.

Probes can vary in length from a few base pairs for synthetic oligonucleotides to larger than one Mbp. Probes of different types can be used to detect distinct DNA types. PCR-amplified repeated DNA sequences, oligonucleotides specific for repeat elements, or cloned repeat elements can be used to detect clusters of repetitive DNA in heterochromatin blocks or centromeric regions of individual chromosomes. These are useful in determining aberrations in the number of chromosomes present in a cell. In contrast, for detecting single locus targets, cDNAs or pieces of cloned genomic DNA, from 100 bp to 1 Mbp in size, can be used.

To detect specific chromosomes or chromosomal regions, chromosome-specific DNA libraries can be used as probes to delineate individual chromosomes from the full chromosomal **complement**. Specific probes have been commercially available for each of the human chromosomes since 1991.

Any given tissue or cell source, such as sections of frozen tumors, imprinted cells, cultured cells, or embedded sections, may be hybridized. The DNA probes are hybridized to chromosomes from dividing (metaphase) or non-dividing (interphase) cells.

The observation of the hybridized sequences is done using epifluorescence microscopy. White light from a source lamp is filtered so that only the relevant wavelengths for excitation of the fluorescent molecules reach the sample. The light emitted by fluorochromes is generally of larger wavelengths, which allows the distinction between excitation and emission light by means of a second optical filter. Therefore, it is possible to see bright-colored signals on a dark background. It is also possible to distinguish between several excitation and emission bands, thus between several fluorochromes, which allows the observation of many different probes on the same target.

FISH has a large number of applications in **molecular biology** and medical science, including **gene** mapping, diagnosis of chromosomal abnormalities, and studies of cellular structure and function. Chromosomes in three-dimensionally preserved nuclei can be "painted" using FISH. In clinical research, FISH can be used for prenatal diagnosis of inherited chromosomal aberrations, postnatal diagnosis of carriers of genetic disease, diagnosis of infectious disease, viral and bacterial disease, tumor cytogenetic diagnosis, and detection of aberrant gene expression. In laboratory research, FISH can be used for mapping chromosomal genes, to study the **evolution** of genomes (Zoo FISH), analyzing nuclear organization, visualization of chromosomal territories and chromatin in interphase cells, to analyze dynamic nuclear processes, somatic hybrid cells, replication, chromosome sorting, and to study tumor biology. It can also be used in developmental biology to study the temporal expression of genes during differentiation and development. Recently, high resolution FISH has become a popular method for ordering genes or DNA markers within chromosomal regions of interest.

See also Biochemical analysis techniques; Biotechnology; Laboratory techniques in immunology; Laboratory techniques in microbiology; Molecular biology and molecular genetics

FLUORESCENCE MICROSCOPY • *see* MICROSCOPE AND MICROSCOPY

FLUORESCENT DYES

The use of fluorescent dyes is the most popular tool for measuring ion properties in living cells. Calcium, magnesium, sodium, and similar species that do not naturally fluoresce can be measured indirectly by complexing them with fluorescent molecules. The use of probes, which fluoresce at one wavelength when unbound, and at a different wavelength when bound to an ion, allows the quantification of the ion level.

Fluorescence has also become popular as an alternative to radiolabeling of peptides. Whereas labeling of peptides with a radioactive compound relies on the introduction of a radiolabeled amino acid as part of the natural structure of the peptide, fluorescent tags are introduced as an additional group to the molecule.

The use of fluorescent dyes allows the detection of minute amounts of the target molecule within a mixture of many other molecules. In combination with light microscopic techniques like confocal laser microscopy, the use of fluorescent dyes allows three-dimensional image constructs to be complied, to provide precise spatial information on the target location. Finally, fluorescence can be used to gain information about phenomena such as blood flow and organelle movement in real time.

The basis of fluorescent dyes relies on the absorption of light at a specific wavelength and, in turn, the excitation of the electrons in the dye to higher energy levels. As the electrons fall back to their lower pre-excited energy levels, they re-emit light at longer wavelengths and so at lower energy levels. The lower-energy light emissions are called spectral shifts. The process can be repeated.

Proper use of a fluorescent dye requires 1) that its use does not alter the shape or function of the target cell, 2) that the dye localizes at the desired location within or on the cell, 3) that the dye maintains its specificity in the presence of competing molecules, and 4) that they operate at near visible wavelengths. Although none of the dyes in use today meets all of these criteria, fluorescent dyes are still useful for staining and observation to a considerable degree.

See also Biochemical analysis techniques; Biotechnology; Electron microscope, transmission and scanning; Electron microscopic examination of microorganisms; Immunofluorescence; Microscope and microscopy

FOOD PRESERVATION

The term food preservation refers to any one of a number of techniques used to prevent food from spoiling. It includes methods such as canning, pickling, drying and freeze-drying, irradiation, **pasteurization**, smoking, and the addition of chemical additives. Food preservation has become an increasingly

important component of the food industry as fewer people eat foods produced on their own lands, and as consumers expect to be able to purchase and consume foods that are out of season.

The vast majority of instances of food spoilage can be attributed to one of two major causes: (1) the attack by pathogens (disease-causing **microorganisms**) such as **bacteria** and molds, or (2) oxidation that causes the destruction of essential biochemical compounds and/or the destruction of plant and animal cells. The various methods that have been devised for preserving foods are all designed to reduce or eliminate one or the other (or both) of these causative agents.

For example, a simple and common method of preserving food is by heating it to some minimum temperature. This process prevents or retards spoilage because high temperatures kill or inactivate most kinds of pathogens. The addition of compounds known as BHA and BHT to foods also prevents spoilage in another different way. These compounds are known to act as antioxidants, preventing chemical reactions that cause the oxidation of food that results in its spoilage. Almost all techniques of preservation are designed to extend the life of food by acting in one of these two ways.

The search for methods of food preservation probably can be traced to the dawn of human civilization. People who lived through harsh winters found it necessary to find some means of insuring a food supply during seasons when no fresh fruits and vegetables were available. Evidence for the use of dehydration (drying) as a method of food preservation, for example, goes back at least 5,000 years. Among the most primitive forms of food preservation that are still in use today are such methods as smoking, drying, salting, freezing, and fermenting.

Early humans probably discovered by accident that certain foods exposed to smoke seem to last longer than those that are not. Meats, fish, fowl, and cheese were among such foods. It appears that compounds present in wood smoke have antimicrobial actions that prevent the growth of organisms that cause spoilage. Today, the process of smoking has become a sophisticated method of food preservation with both hot and cold forms in use. Hot smoking is used primarily with fresh or frozen foods, while cold smoking is used most often with salted products. The most advantageous conditions for each kind of smoking—air velocity, relative humidity, length of exposure, and salt content, for example—are now generally understood and applied during the smoking process. For example, electrostatic precipitators can be employed to attract smoke particles and improve the penetration of the particles into meat or fish. So many alternative forms of preservation are now available that smoking no longer holds the position of importance it once did with ancient peoples. More frequently, the process is used to add interesting and distinctive flavors to foods.

Because most disease-causing organisms require a moist environment in which to survive and multiply, drying is a natural technique for preventing spoilage. Indeed, the act of simply leaving foods out in the sun and wind to dry out is probably one of the earliest forms of food preservation. Evidence for the drying of meats, fish, fruits, and vegetables go back to the earliest recorded human history. At some point, humans also learned that the drying process could be hastened and improved by various mechanical techniques. For example, the Arabs learned early on that apricots could be preserved almost indefinitely by macerating them, boiling them, and then leaving them to dry on broad sheets. The product of this technique, quamaradeen, is still made by the same process in modern Muslim countries.

Today, a host of dehydrating techniques are known and used. The specific technique adopted depends on the properties of the food being preserved. For example, a traditional method for preserving rice is to allow it to dry naturally in the fields or on drying racks in barns for about two weeks. After this period of time, the native rice is threshed and then dried again by allowing it to sit on straw mats in the sun for about three days. Modern drying techniques make use of fans and heaters in controlled environments. Such methods avoid the uncertainties that arise from leaving crops in the field to dry under natural conditions. Controlled temperature air drying is especially popular for the preservation of grains such as maize, barley, and bulgur.

Vacuum drying is a form of preservation in which a food is placed in a large container from which air is removed. Water vapor pressure within the food is greater than that outside of it, and water evaporates more quickly from the food than in a normal atmosphere. Vacuum drying is biologically desirable since some **enzymes** that cause oxidation of foods become active during normal air drying. These enzymes do not appear to be as active under vacuum drying conditions, however. Two of the special advantages of vacuum drying are that the process is more efficient at removing water from a food product, and it takes place more quickly than air drying. In one study, for example, the drying time of a fish fillet was reduced from about 16 hours by air drying to six hours as a result of vacuum drying.

Coffee drinkers are familiar with the process of dehydration known as spray drying. In this process, a concentrated solution of coffee in water is sprayed though a disk with many small holes in it. The surface area of the original coffee grounds is increased many times, making dehydration of the dry product much more efficient. Freeze-drying is a method of preservation that makes use of the physical principle known as sublimation. Sublimation is the process by which a solid passes directly to the gaseous phase without first melting. Freeze-drying is a desirable way of preserving food because at low temperatures (commonly around 14°F to –13°F [–10°C to –25°C]) chemical reactions take place very slowly and pathogens have difficulty surviving. The food to be preserved by this method is first frozen and then placed into a vacuum chamber. Water in the food first freezes and then sublimes, leaving a moisture content in the final product of as low as 0.5%.

The precise mechanism by which salting preserves food is not entirely understood. It is known that salt binds with water molecules and thus acts as a dehydrating agent in foods. A high level of salinity may also impair the conditions under which pathogens can survive. In any case, the value of adding salt to foods for preservation has been well known for centuries. Sugar appears to have effects similar to those of salt in preventing spoilage of food. The use of either compound (and of certain

other natural materials) is known as curing. A desirable side effect of using salt or sugar as a food preservative is, of course, the pleasant flavor each compound adds to the final product.

Curing can be accomplished in a variety of ways. Meats can be submerged in a salt solution known as brine, for example, or the salt can be rubbed on the meat by hand. The injection of salt solutions into meats has also become popular. Food scientists have now learned that a number of factors relating to the food product and to the preservative conditions affect the efficiency of curing. Some of the food factors include the type of food being preserved, the fat content, and the size of treated pieces. Preservative factors include brine temperature and concentration, and the presence of impurities.

Curing is used with certain fruits and vegetables, such as cabbage (in the making of sauerkraut), cucumbers (in the making of pickles), and olives. It is probably most popular, however, in the preservation of meats and fish. Honey-cured hams, bacon, and corned beef ("corn" is a term for a form of salt crystals) are common examples.

Freezing is an effective form of food preservation because the pathogens that cause food spoilage are killed or do not grow very rapidly at reduced temperatures. The process is less effective in food preservation than are thermal techniques such as boiling because pathogens are more likely to be able to survive cold temperatures than hot temperatures. In fact, one of the problems surrounding the use of freezing as a method of food preservation is the danger that pathogens deactivated (but not killed) by the process will once again become active when the frozen food thaws.

A number of factors are involved in the **selection** of the best approach to the freezing of foods, including the temperature to be used, the rate at which freezing is to take place, and the actual method used to freeze the food. Because of differences in cellular composition, foods actually begin to freeze at different temperatures ranging from about 31°F (–0.6°C) for some kinds of fish to 19°F (–7°C) for some kinds of fruits.

The rate at which food is frozen is also a factor, primarily because of aesthetic reasons. The more slowly food is frozen, the larger the ice crystals that are formed. Large ice crystals have the tendency to cause rupture of cells and the destruction of texture in meats, fish, vegetables, and fruits. In order to deal with this problem, the technique of quick-freezing has been developed. In quick-freezing, a food is cooled to or below its freezing point as quickly as possible. The product thus obtained, when thawed, tends to have a firm, more natural texture than is the case with most slow-frozen foods.

About a half dozen methods for the freezing of foods have been developed. One, described as the plate, or contact, freezing technique, was invented by the American inventor Charles Birdseye in 1929. In this method, food to be frozen is placed on a refrigerated plate and cooled to a temperature less than its freezing point. Alternatively, the food may be placed between two parallel refrigerated plates and frozen. Another technique for freezing foods is by immersion in very cold liquids. At one time, sodium chloride brine solutions were widely used for this purpose. A 10% brine solution, for example, has a freezing point of about 21°F (–6°C), well within the desired freezing range for many foods. More recently, liquid nitrogen has been used for immersion freezing. The temperature of liquid nitrogen is about –320°F (–195.5°C), so that foods immersed in this substance freeze very quickly.

As with most methods of food preservation, freezing works better with some foods than with others. Fish, meat, poultry, and citrus fruit juices (such as frozen orange juice concentrate) are among the foods most commonly preserved by this method.

Fermentation is a naturally occurring chemical reaction by which a natural food is converted into another form by pathogens. It is a process in which food spoils, but results in the formation of an edible product. Perhaps the best example of such a food is cheese. Fresh milk does not remain in edible condition for a very long period of time. Its **pH** is such that harmful pathogens begin to grow in it very rapidly. Early humans discovered, however, that the spoilage of milk can be controlled in such a way as to produce a new product, cheese.

Bread is another food product made by the process of fermentation. Flour, water, sugar, milk, and other raw materials are mixed together with yeasts and then baked. The addition of yeasts brings about the fermentation of sugars present in the mixture, resulting in the formation of a product that will remain edible much longer than will the original raw materials used in the bread-making process.

Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water. In this respect, heating foods is a form of food preservation comparable to that of freezing but much superior to it in its effectiveness. A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens.

In many cases, foods are actually cooked prior to their being packaged and stored. In other cases, cooking is neither appropriate nor necessary. The most familiar example of the latter situation is pasteurization. During the 1860s, the French bacteriologist **Louis Pasteur** discovered that pathogens in foods could be destroyed by heating those foods to a certain minimum temperature. The process was particularly appealing for the preservation of milk since preserving milk by boiling is not a practical approach. Conventional methods of pasteurization called for the heating of milk to a temperature between 145 and 149°F (63 and 65°C) for a period of about 30 minutes, and then cooling it to room temperature. In a more recent revision of that process, milk can also be "flash-pasteurized" by raising its temperature to about 160°F (71°C) for a minimum of 15 seconds, with equally successful results. A process known as ultra-high-pasteurization uses even higher temperatures, of the order of 194–266°F (90–130°C), for periods of a second or more.

One of the most common methods for preserving foods today is to enclose them in a sterile container. The term "canning" refers to this method although the specific container can be glass, plastic, or some other material as well as a metal can, from which the procedure originally obtained its name. The basic principle behind canning is that a food is sterilized, usually by heating, and then placed within an air-tight container. In the absence of air, no new pathogens can gain access to the

sterilized food. In most canning operations, the food to be packaged is first prepared in some way—cleaned, peeled, sliced, chopped, or treated in some other way—and then placed directly into the container. The container is then placed in hot water or some other environment where its temperature is raised above the boiling point of water for some period of time. This heating process achieves two goals at once. First, it kills the vast majority of pathogens that may be present in the container. Second, it forces out most of the air above the food in the container.

After heating has been completed, the top of the container is sealed. In home canning procedures, one way of sealing the (usually glass) container is to place a layer of melted paraffin directly on top of the food. As the paraffin cools, it forms a tight solid seal on top of the food. Instead of or in addition to the paraffin seal, the container is also sealed with a metal screw top containing a rubber gasket. The first glass jar designed for this type of home canning operation, the Mason jar, was patented in 1858.

The commercial packaging of foods frequently makes use of tin, aluminum, or other kinds of metallic cans. The technology for this kind of canning was first developed in the mid-1800s, when individual workers hand-sealed cans after foods had been cooked within them. At this stage, a single worker could seldom produce more than 100 "canisters" (from which the word "can" later came) of food a day. With the development of far more efficient canning machines in the late nineteenth century, the mass production of canned foods became a reality.

As with home canning, the process of preserving foods in metal cans is simple in concept. The foods are prepared and the empty cans are sterilized. The prepared foods are then added to the sterile metal can, the filled can is heated to a sterilizing temperature, and the cans are then sealed by a machine. Modern machines are capable of moving a minimum of 1,000 cans per minute through the sealing operation.

The majority of food preservation operations used today also employ some kind of chemical additive to reduce spoilage. Of the many dozens of chemical additives available, all are designed either to kill or retard the growth of pathogens or to prevent or retard chemical reactions that result in the oxidation of foods. Some familiar examples of the former class of food additives are sodium benzoate and benzoic acid; calcium, sodium propionate, and propionic acid; calcium, potassium, sodium sorbate, and sorbic acid; and sodium and potassium sulfite. Examples of the latter class of additives include calcium, sodium ascorbate, and ascorbic acid (vitamin C); butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); lecithin; and sodium and potassium sulfite and sulfur dioxide.

A special class of additives that reduce oxidation is known as the sequestrants. Sequestrants are compounds that "capture" metallic ions, such as those of copper, iron, and nickel, and remove them from contact with foods. The removal of these ions helps preserve foods because in their free state they increase the rate at which oxidation of foods takes place. Some examples of sequestrants used as food preservatives are ethylenediamine-tetraacetic acid (EDTA), citric acid, sorbitol, and tartaric acid.

The lethal effects of radiation on pathogens has been known for many years. Since the 1950s, research in the United States has been directed at the use of this technique for preserving certain kinds of food. The radiation used for food preservation is normally gamma radiation from radioactive isotopes or machine-generated x rays or electron beams. One of the first applications of radiation for food preservation was in the treatment of various kinds of herbs and spices, an application approved by the U.S. Food and Drug Administration (FDA) in 1983. In 1985, the FDA extended its approval to the use of radiation for the treatment of pork as a means of destroying the pathogens that cause trichinosis. Experts predict that the ease and efficiency of food preservation by means of radiation will develop considerably in the future. That future is somewhat clouded, however, by fears expressed by some scientists and members of the general public about the dangers that irradiated foods may have for humans. In addition to a generalized concern about the possibilities of being exposed to additional levels of radiation in irradiated foods (not a possibility), critics have raised questions about the creation of new and possibly harmful compounds in food that has been exposed to radiation.

See also Biotechnology; Botulism; Food safety; History of microbiology; History of public health; Salmonella food poisoning; Winemaking

FOOD SAFETY

Food is a source of nutrients not only to humans but to **microorganisms** as well. The organic compounds and moisture that are often present in foods present an ideal environment for the growth of various microorganisms. The monitoring of the raw food and of any processing steps required prior to the consumption of the food are necessary to prevent transmission of disease-causing microorganisms from the food to humans.

Bacteria, **viruses**, **parasites**, and toxin by-products of microorganisms, chemicals, and heavy metals can cause foodborne maladies. These agents are responsible for over 200 different foodborne diseases. In the United States alone, foodborne diseases cause an estimated 75 million illnesses every year, and 7,000 to 9,000 deaths.

Aside from the human toll, the economic consequences of foodborne illnesses are considerable. In 1988, for example, human foodborne diarrheal disease in the United States cost the U.S. economy an estimated five to seven billion dollars in medical care and lost productivity.

The threat from foodborne disease causing agents is not equal. For example, the Norwalk-like viruses cause approximately 9 million illnesses each year, but the fatality rate is only 0.001%. *Vibrio vulnificus* causes fewer than 50 cases each year but almost 40% of those people die. Finally, the bacteria *Salmonella, Listeria monocytogenes*, and *Toxoplasma gondii* cause only about 20% of the total cases but are responsible for almost 80% of the total deaths from foodborne illnesses.

Raw oysters can harbor microbial toxins.

The **Centers for Disease Control** data has demonstrated that *Campylobacter jejuni* is the leading cause of foodborne illness in the United States. Another bacteria, *Salmonella* is the next leading cause. The third cause of foodborne illness is the bacterium ***Escherichia coli*** O157:H7. Poultry and ground meat are prime targets for bacterial **contamination**. Indeed, monitoring studies have demonstrated that some 70–90% of poultry carry *Campylobacter jejuni*.

Food safety needs to consider the influences of the microbial pathogen, the human host and the exposure of the food to the environment that promotes contamination. The environment can include the physical parameters such as the temperature, moisture, or other such factors. As well the environment can be the site of the foodstuff, such as the farmyard or the processing plant. Ensuring safety of food from microbial threat must consider all three of the influences. For example, reducing the length of time that a food is exposed to a questionable environment, but doing nothing to remove microbes from the environment only slightly reduces the risk of food contamination. Significant protection of foods depends on reducing the risk from the environment, microorganism of interest and of the human host.

The treatment of foods prior to consumption is a vital factor in ensuring food safety. Some of these treatments have been known for a long time. Salting of meats and drying of foods on long sea voyages was practiced several centuries ago, for example. The canning of foods began in the eighteenth century. Within the last 150 years, the link between hygienic conditions and the quality and safety of foods was recognized. Some of the advances in food safety arose from the need for foods on long military campaigns, such as those undertaken by Napoleon in the nineteenth century. Also, advances were spurred by the demands of the nascent food industry. As the distance between the farm and the market began to grow larger, and the shipping of food became more commonplace, the problems of food contamination became evident. Practices to render food safe for shipping, storage and subsequent consumption were necessary if the food industry was to grow and flourish.

The heat treatment of milk known as **pasteurization** began in the 1890s. Pasteurization is the transient exposure of milk to temperatures high enough to kill microbes, while preserving the taste and visual quality of the milk. Milk is now routinely pasteurized before sale to kill any bacteria that would otherwise growth in the wonderful growth medium that the liquid provides. Within the past thirty years the use of radiation to kill microbes in food has been utilized. While a very effective method to ensure food safety, irradiation is still subject to consumer uncertainty, which has to date limited its usefulness. As a final example, within the past two decades, the danger posed by intestinal bacterial pathogens, particularly *Escherichia coli* O157:H7 has resulted in the heightened recognition of the need for proper food preparation and personal **hygiene**.

Food safety is also dependent on the development and enforcement of standards of food preparation, handling and inspection. Often the mandated inspection of foods requires the food to be examined in certain ways and to achieve set benchmarks of quality (such as the total absence of fecal coliform bacteria). Violation of the quality standards can result in the immediate shut down of the food processing operation until the problem is located and rectified.

Most of the food safety legislation and inspection efforts are aimed at the processing of food. It is difficult to monitor the home environment and to enforce codes of hygiene there. Yet, food safety in the home is of paramount importance. The improper storage of foods prepared with raw or undercooked eggs, for example, can lead to the growth of microorganisms in the food. Depending on the microbe and whether toxins are produced, food poisoning or food intoxication can result from eating the food dish. Additionally, improper cleaning of cutting and other preparation surfaces can lead to the cross-contamination of one food by another. Good hygienic practices are as important in the home as on the farm, in the feedlot, and in the processing plant.

See also BSE and CJD disease; BSE and CJD disease, advances in research; BSE and CJD disease, ethical issues and socio-economic impact; Enterotoxin and exotoxin; Food preservation; Transmission of pathogens

Destruction of sheep to prevent the spread of infection during an outbreak of foot-and-mouth disease.

FOOT-AND-MOUTH DISEASE

Often inaccurately called hoof-and-mouth disease, this highly contagious virus causes blisters in the mouth and on the feet surrounding hoofs of animals with cleft, or divided hoofs, such as sheep, cattle and hogs. The disease was first noted in Europe in 1809; the first outbreak in the United States came in 1870. Although it seldom spreads to humans, it can be transmitted through contaminated milk or the handling of infected animals.

Outbreaks are expensive for the animal owners who must kill the infected animals and incinerate or bury or them in quicklime. Then the animals' living quarters are disinfected, while feed and litter are burned. The farm is quarantined by state and federal officials who can decide to extend the quarantine to the general area or the whole state. Friedrich August Löffler (1852–1915), a German bacteriologist who discovered the bacillus of **diphtheria** in 1884, also demonstrated in 1898 that a virus causes hoof-and-mouth disease. It was the first time a virus was reported to be the cause of an animal disease.

An infected animal can take up to four days to begin showing symptoms of fever, smacking of lips and drooling. Eventually, blisters appear on the mouth, tongue and inside of the lips and the animal becomes lame just before blisters appear in the hoof area.

Löffler, working with Dr. Paul Frosch (1860–1928), a veterinary bacteriologist, extracted lymph from the blisters on the mouths and udders of diseased cattle. The lymph was diluted in sterile water and passed through filters. The researchers expected the filtrate to be an antitoxin of foot-and-mouth disease similar to the one for **smallpox**.

But Löffler and Frosch were wrong; when the filtrates were injected into healthy animals, they became sick. Therefore, they concluded the causative agent was not a bacterial toxin, but instead was a non-toxin producing bacterium too small to be seen under the **microscope**, yet small enough to pass through the filters. It wasn't until 1957 that scientists were able to get their first look at the causative agent, one of the smallest **viruses** to cause an animal disease.

In February, 2001, a devastating outbreak of foot-and-mouth disease began among the stock of England's pig, sheep, and cattle ranchers. Epidemiologists (investigators in infectious disease) determined that the outbreak began in a swill (garbage) feeding farm in one county, and spread first by the wind to a nearby sheep farm, then by sheep markets to farms across the English countryside. Even before the outbreak was detected, the virus had infected livestock in 43 farms. Despite massive quarantining and culling of herds (over 4 million animals were destroyed), by the time the outbreak was contained almost a year later, the disease had spread to areas of Ireland, France, and the Netherlands.

English citizens lost billions of dollars worth of income as markets for English meat and dairy products evaporated,

animals were decimated, and tourists avoided the English countryside. Use of an available **vaccine** to attempt to curb the epidemic was rejected by most scientists, as the virus incubation time was short (often less than 72 hours), and the **immunity** gained from the vaccine was short-lived. Meanwhile, the Unites States and other countries adopted inclusive measures to prevent the importation of the foot-and-mouth virus, from carefully restricting the importation of animal products, to the sanitizing of shoes of airplane passengers arriving in the U.S. from England. As of April 2002, the outbreak continued to be contained, with the last confirmed foot-and-mouth case in England occurring six months prior at a farm in Northumberland, and the restoration of "Foot-and-mouth-Free" status restored to livestock herds of the United Kingdom by the World Organization for Animal Health (Office Internationale des Epizooties).

See also Animal models of infection; Epidemics, viral; Epidemiology, tracking diseases with technology; Epidemiology; Veterinary microbiology

FORENSIC IDENTIFICATION OF MICROORGANISMS

• *see* GENETIC IDENTIFICATION OF MICROORGANISMS

FORENSIC IMMUNOLOGY AND BACTERIOLOGY

• *see* GENETIC IDENTIFICATION OF MICROORGANISMS

FOSSILIZATION OF BACTERIA

Studies of fossilization of **bacteria** provide an indication of the age of ancient bacteria. Fossils of cyanobacteria or "blue-green algae" have been recovered from rocks that are nearly 3.5 million years old. Bacteria known as magnetobacteria form very small crystals of a magnetic compound inside the cells. These crystals have been found inside rock that is two billion years old.

The fossilization process in cyanobacteria and other bacteria appears to depend on the ability of the bacteria to trap sediment and metals from the surrounding solution. Cyanobacteria tend to grow as mats in their aquatic environment. The mats can retain sediment. Over time and under pressure the sediment entraps the bacteria in rock. As with other living organisms, the internal structure of such bacteria is replaced by minerals, notably pyrite or siderite (iron carbonate). The result, after thousands to millions of years, is a replica of the once-living cell.

Other bacteria that elaborate a carbohydrate network around themselves also can become fossilized. The evidence for this type of fossilization rests with laboratory experiments where bacteria are incubated in a metal-containing solution under conditions of temperature and pressure that attempt to mimic the forces found in geological formations. Experiments with *Bacillus subtilis* demonstrated that the bacteria act as a site of precipitation for silica, the ferric form of iron, and of elemental gold. The binding of some of the metal ions to available sites within the carbohydrate network then acts to drive the precipitation of unstable metals out of solution and onto the previously deposited metal. The resulting cascade of precipitation can encase the entire bacterium in metallic species. On primordial Earth, this metal binding may have been the beginning of the fossilization process.

The deposition of metals inside carbohydrate networks like the capsule or exopolysaccharide surrounding bacteria is a normal feature of **bacterial growth**. Indeed, metal deposition can change the three-dimensional arrangement of the carbohydrate strands so as to make the penetration of antibacterial agents through the matrix more difficult. In an environment—such as occurs in the lungs of a cystic fibrosis patient— this micro-fossilization of bacteria confers a survival advantage to the cells.

In contrast to fossils of organisms such as dinosaurs, the preservation of internal detail of **microorganisms** seldom occurs. Prokaryotes have little internal structure to preserve. However, the mere presence of the microfossils is valuable, as they can indicate the presence of microbial life at that point in geological time.

Bacteria have been fossilized in amber, which is fossilized tree resin. Several reports have described the resuscitation of bacteria recovered from amber as well as bacteria recovered from a crystal in rock that is millions of years old. Although these claims have been disputed, a number of microbiologists assert that the care exercised by the experimenters lends increases the validity of their studies.

In the late 1990s a meteorite from the planet Mars was shown to contain bodies that appeared similar to bacterial fossils that have been found in rocks on Earth. Since then, further studies have indicated that the bodies may have arisen by inorganic (non-living) processes. Nonetheless, the possibility that these bodies are the first extraterrestrial bacterial fossils has not been definitively ruled out.

See also Bacterial surface layers; Biogeochemical cycles; Glycocalyx

FRIEND, CHARLOTTE (1921-1987)

American microbiologist

As the first scientist to discover a direct link between **viruses** and cancer, Charlotte Friend made important breakthroughs in cancer research, particularly that of leukemia. She was successful in immunizing mice against leukemia and in pointing a way toward new methods of treating the disease. Because of Friend's work, medical researchers developed a greater understanding of cancer and how it can be fought.

Friend was born on March 11, 1921, in New York City to Russian immigrants. Her father died of endocarditis (heart **inflammation**) when Charlotte was three, a factor that may have influenced her early decision to enter the medical field; at age ten she wrote a school composition entitled, "Why I Want to Become a Bacteriologist." Her mother's job as a pharmacist

also exposed Friend to medicine. After graduating from Hunter College in 1944, she immediately enlisted in the U.S. Navy during World War II, rising to the rank of lieutenant junior grade.

After the war, Friend entered graduate school at Yale University, obtaining her Ph.D. in bacteriology in 1950. Soon afterward, she was hired by the Memorial Sloan-Kettering Institute for Cancer Research, and in 1952, became an associate professor in microbiology at Cornell University, which had just set up a joint program with the institute. During that time, Friend became interested in cancer, particularly leukemia, a cancer of blood-forming organs that was a leading killer of children. Her research on the cause of this disease led her to believe that, contrary to the prevailing medical opinion, leukemia in mice is caused by a virus. To confirm her theory, Friend took samples of leukemia tissue from mice and, after putting the material through a filter to remove cells, injected it into healthy mice. These animals developed leukemia, indicating that the cause of the disease was a substance smaller than a cell. Using an **electron microscope**, Friend was able to discover and photograph the virus she believed responsible for leukemia.

However, when Friend presented her findings at the April 1956, annual meeting of the American Association for Cancer Research, she was denounced by many other researchers, who refused to believe that a virus was responsible for leukemia. Over the next year support for Friend's theory mounted, first as Dr. Jacob Furth declared that his experiments had confirmed the existence of such a virus in mice with leukemia. Even more importantly, Friend was successful in vaccinating mice against leukemia by injecting a weakened form of the virus (now called the "Friend virus") into healthy mice, so they could develop antibodies to fight off the normal virus. Friend's presentation of a paper on this **vaccine** at the cancer research association's 1957 meeting succeeded in laying to rest the skepticism that had greeted her the previous year.

In 1962, Friend was honored with the Alfred P. Sloan Award for Cancer Research and another award from the American Cancer Society for her work. The next year she became a member of the New York Academy of Sciences, an organization that has members from all fifty states and more than eighty countries. In 1966, Friend left Sloan-Kettering to become a professor and director at the Center for Experimental Cell Biology at the newly formed medical school of New York's Mount Sinai Hospital. During this time, she continued her research on leukemia, and in 1972, she announced the discovery of a method of altering a leukemia mouse cell in a test tube so that it would no longer multiply. Through chemical treatment, the malignant red blood cell could be made to produce hemoglobin, as do normal cells.

Although the virus responsible for leukemia in mice has been discovered, there is no confirmation that a virus causes leukemia in humans. Likewise, her treatment for malignant red blood cells has limited application, because it will not work outside of test tubes. Nonetheless, Friend had pointed out a possible cause of cancer and developed a first step toward fighting leukemia (and possibly other cancers) by targeting specific cells.

In 1976, Friend was elected president of the American Association for Cancer Research, the same organization whose members had so strongly criticized her twenty years earlier. Two years later, she was chosen the first woman president of the New York Academy of Sciences. Friend was long active in supporting other women scientists and in speaking out on women's issues. During her later years, she expressed concern over the tendency to emphasize patient care over basic research, feeling that without sufficient funding for research, new breakthroughs in patient care would be impossible. Friend died on January 13, 1987, of lymphoma.

Charlotte Friend, an important cancer researcher.

See also Viral vectors in gene therapy; Virology; Virus replication; Viruses and responses to viral infection

FUME HOOD

A fume hood is an enclosed work space in a laboratory that prevents the outward flow of air. Fume hoods cab be designed for work with inorganic or radioactive materials, or with biological materials. Biological fume hoods can be equipped with filters, to ensure that the air entering and exiting the cabinet is sterile. This minimizes the risk of exposure of laboratory personnel to biological agents that could be a health threat. Also, the work surfaces and materials inside the fume hood are protected from

contamination from airborne **bacteria** or **viruses**. The latter is of particular relevance in some viral research, where the tissue surfaces used to grow the virus are prone to contamination.

The design of fume hoods differs, depending on the intended purpose (general purpose, chemical, radioisotope, biological). But all fume hoods share the feature of an inward flow of air. In biological fume hoods the flow of sterile air is typically from the back of the cabinet toward the laboratory worker, and from the top of the fume hood downward across the opening at the front of the hood. This pattern of airflow ensures that any **microorganisms** residing on the laboratory worker are not carried into the work surface, and that no air from inside the cabinet escapes to the outside of the cabinet. Any air that is exhausted back into the laboratory first passes through filters that are designed to remove biological and viral contaminants. The most popular type of biological filter is the high-energy particulate air (or HEPA) filter.

Biological fume hoods can have a moveable, protective glass partition at the front. Most hoods also have a gas source inside, so that sterile work, such as the flaming of inoculation loops, can be done. The fume hood should be positioned in an area of the laboratory where there is less traffic back and forth, which lessens the turbulence of air outside the fume hood.

The filtering system of biological fume hoods restricts its use to biological work. Work involving noxious chemicals and vapors needs to be conducted in another, specially designed chemical fume hood.

The construction of fume hoods is conducted according to strict protocols of safety and performance monitoring. In normal laboratory use, the continued performance of a fume hood is regularly monitored and test results recorded. Often such checks are a mandatory requirement of the ongoing certification of an analysis laboratory. Accordingly, laboratories must properly maintain and use fume hoods to continue to meet operating rules and regulations.

See also Bioterrorism, protective measures; Containment and release prevention protocol

FUNGAL GENETICS

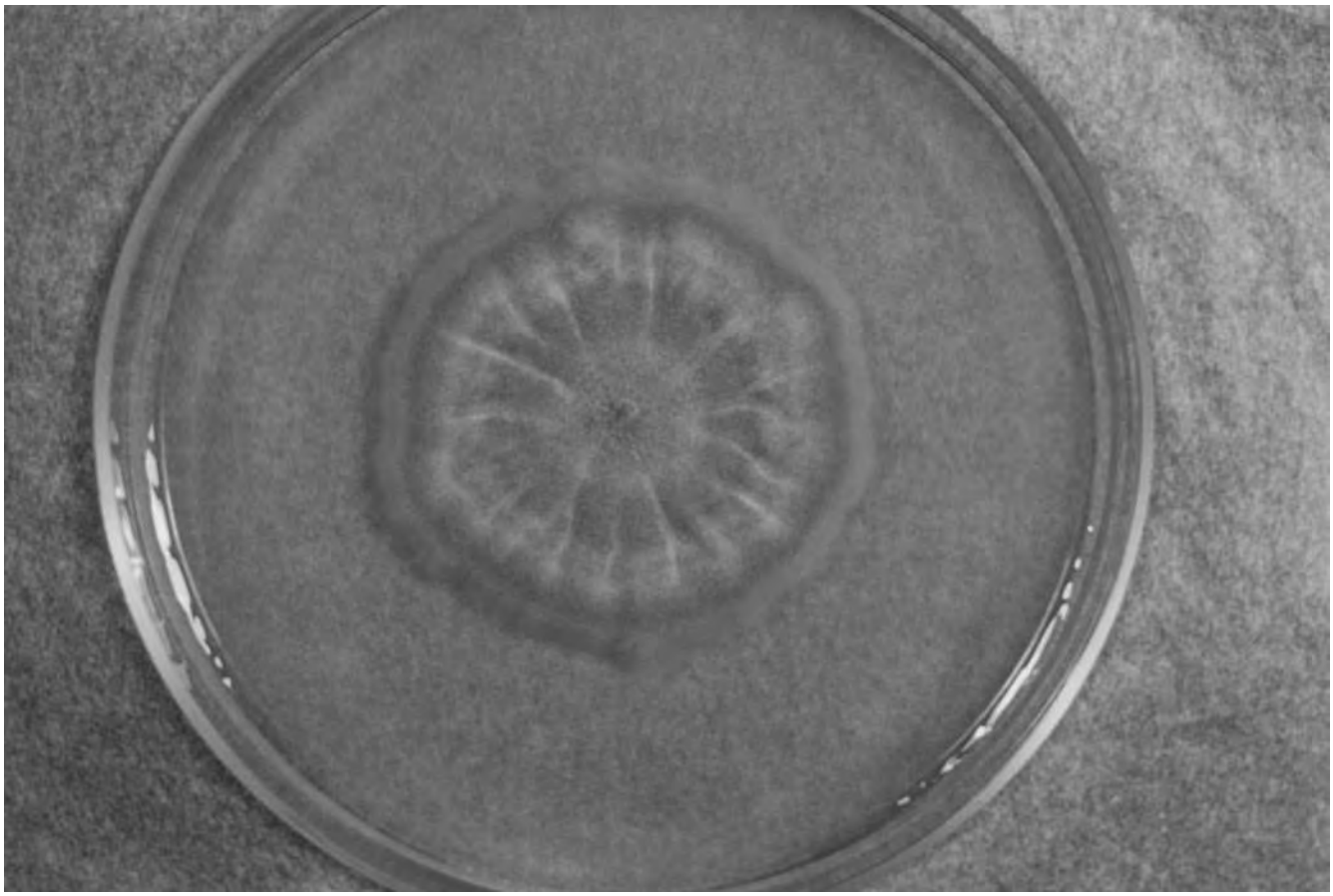
Fungi possess strikingly different morphologies. They include large, fleshy, and often colorful mushrooms or toadstools, filamentous organisms only just visible to the naked eye, and single-celled organisms such as yeasts. Molds are important agents of decay. They also produce a large number of industrially important compounds like **antibiotics** (**penicillin**, griseofulvin, etc.), organic acids (citric acid, gluconic acid, etc.), **enzymes** (alpha-amylases, lipase, etc.), traditional foods (softening and flavoring of cheese, shoyu soy sauce, etc.), and a number of other miscellaneous products (gibberellins, ergot alkaloids, steroid bioconversions). As late as 1974 the only widely applicable techniques for strain improvement were mutation, screening, and **selection**. While these techniques proved dramatically successful in improving penicillin production, they deflected attempts to employ a more sophisticated approach to genetic manipulation. The study of fungal genetics has recently changed beyond all recognition.

The natural genetic variation present in fungal species has been characterized using molecular methods such as electrophoretic karyotyping, restriction fragment length polymorphism, **DNA** finger printing, and DNA sequence comparisons. The causes for the variation include chromosomal polymorphism, changes in repetitive DNA, **transposons**, virus-like elements, and mitochondrial **plasmids**.

Genetic **recombination** occurs naturally in many fungi. Many industrially important fungi such as *Aspergilli* and *Penicillia* lack sexuality, so in these species parasexual systems (cycles) provide the basis for genetic study and breeding programs. The parasexual cycle is a series of events that can be induced when two genetically different strains are grown together in the laboratory. A heterokaryon, which is **mycelium** with two different nuclei derived from two different haploid strains, is produced by the fusion of **hyphae**. Increased penicillin titer in the haploid progeny of parasexual crosses has been achieved in *Penicillium chrysogenum*. A more direct approach has been developed using **protoplasts**. These are isolated from vegetative cells of fungi or yeasts by removing the cell wall by digestion using a cell wall degrading enzyme. Protoplasts from the two strains can be fused by treatment with polyethylene glycol. Protoplast fusion in fungi initiates the parasexual cycle, resulting in the formation of diploidy and mitotic recombination and segregation. A selection procedure to screen such fusants is done using genetic markers. A good example of applying this technique is the fusion of a fast growing but poor glucoamylase producer with a slow growing but excellent producer of glucoamylase. The desired result will be a strain that is both fast growing and an excellent producer of enzyme.

The realization that **transformation** of genetic material into fungi can occur came with the discovery that yeasts like ***Saccharomyces cerevisiae*** and filamentous fungi like *Podospora anserine* contain **plasmids**. Currently transformation technology is largely based on the use of *Neurospora crassa* and *Aspergillus nidulans,* though methods for use in filamentous organisms are being developed. The protocols used in transformation of filamentous fungi involve **cloning** the desired **gene** into the plasmid from ***E. coli*** or a plasmid constructed from genetic material from *E. coli* and *Saccharomyces cerevisiae*. Protoplasts from the recipient strains are then formed and mixed with the plasmid. After incubating for a short time to allow for the uptake of the plasmid DNA, the protoplasts are allowed to regenerate and the cells are screened for the presence of the specific marker.

The application of recombinant DNA to yeasts and filamentous fungi has opened up new possibilities in relation to the construction of highly productive strains. The filamentous fungi are now established as potent host organisms for the production of heterologous proteins. This is particularly useful as expression of specific proteins can reach relatively high levels. Using *Aspergillus* as a host for reproduction has led to the production of many recombinant products like human therapeutic proteins, including growth factors, **cytokines**, and hormones. While expression can be good in *E. coli,* lack of posttranslational modifications has limited their usage. The use of



Fungus colony.

Saccharomyces species has not been highly successful for the production of extracellular proteins. Most of the initial advances for the production of heterologous proteins has been with filamentous fungi, namely *Aspergillus nidulans*. Although this organism is not of industrial importance it is nevertheless genetically well characterized; in addition, this organism has secretion signals that result in recombinant proteins being identical to mammalian cells. This allows the product from such systems to be used safely in human therapy. Other systems that have been used include Pichia and Trichoderma, which have been widely used in industry. Now that the complete genome of *S. cerevisiae* has been deciphered, and with more fungi genomes in the pipeline, an even better understanding of fungal genetics is certain.

See also Cell cycle (Eukaryotic), genetic regulation of; Microbial genetics

FUNGI

Fungi play an essential role in breaking down organic matter and thereby allowing nutrients to be recycled in nature. As such, they are important decomposers and without them living communities would become buried in their own waste. Some fungi, the saprobes, get their nutrients from nonliving organic matter, such as dead plants and animal wastes, clothing, paper, leather, and other materials. Others, the **parasites**, get nutrients from the tissues of living organisms. Both types of fungi obtain nutrients by secreting **enzymes** from their cells that break down large organic molecules into smaller components. The fungi cells can then absorb the nutrients.

Although the term fungus invokes unpleasant images for some people, fungi are a source of **antibiotics**, vitamins, and industrial chemicals. **Yeast**, a kind of fungi, is used to ferment bread and alcoholic beverages. Nevertheless, fungi also cause athlete's foot, yeast infections, food spoilage, wheat and corn diseases, and, perhaps most well known, the Irish potato famine of 1843–1847 (caused by the fungus *Phytophthora infestans*), which contributed to the deaths of 250,000 people in Ireland.

Fungi are not plants, and are unique and separate forms of life that are classified in their own kingdom. Approximately 75,000 species of fungi have been described, and scientists estimate that more than 90% of all fungi species on the planet have yet to be discovered. The fungi body, called **mycelium**, is composed of threadlike filaments called **hyphae**. All fungi can

reproduce asexually by cell division, budding, fragmentation, or spores, although some reproduce sexually.

The main groups of fungi are chytrids, water molds, zygosporangium-forming fungi, sac fungi, and club fungi. Chyrids live in muddy or aquatic habitats and feed on decaying plants, though some live as parasites on living plants, animals, and other fungi. Water molds, distantly related to other fungi, play an important role as decomposers in aquatic habitats. Some, however, live as parasites on aquatic animals and terrestrial plants, including potato plants that can be destroyed by certain types of water molds. Zygosporangium-forming fungi also can be either saprobes, such as the well-known black bread **mold**, or parasites on insects, such as houseflies. Sac fungi, of which more than 30,000 species are known, include the yeast used to leaven bread and alcoholic beverages. However, many of these fungi also cause diseases in plants. Club fungi, numbering more than 25,000 species, include mushrooms, stinkhorns, and puffballs. While some fingi are edible, others produce deadly poisons.

See also Candidiasis; Chitin; Fermentation; Fungal genetics; History of the development of antibiotics; Lichens; Winemaking

FUNGICIDES

Fungicides are chemicals that inhibit the growth of **fungi**. Fungi can attack agricultural crops, garden plants, wood and wood products (dry rot in particular is a major problem), and many other items of use to humans. Fungicides usually kill the fungus that is causing the damage. Sulfur, sulfur-containing compounds, organic salts of iron, and heavy metals are all used as fungicides. Other fungicide types include carbamates or thiocarbamates such as benomyl and ziram, thiozoles such as etridiazole, triazines such as anilazine, and substituted organics such as chlorothalonil. Many non-drug fungicides have low mammalian tolerance for toxicity, and have been shown to cause cancer or reproductive toxicity in experimental animal studies.

Fungicides operate in different ways depending upon the species that they are designed to combat. Many are poisons and their application must be undertaken carefully or over-application may kill other plants in the area. Some fungicides disrupt some of the metabolic pathways of fungi by inhibiting energy production or biosynthesis, and others disrupt the fungal cell wall, which is made of **chitin**, as opposed to the cellulose of plant cell walls. Chitin is a structural polysaccharide and is composed of chains of N-acetyl-D-glucosamine units. Fungal pathogens come from two main groups of fungi, the ascomycetes (rusts and smuts) and the basidiomycetes (the higher fungi—mushrooms, toadstools, and bracket fungi).

Human fungal infections, such as athlete's foot, can be treated by fungicides normally referred to as antifungal agents or antimycotics. Compounds such as fluconazole, clotrimazole, and nystatin are used to treat human fungal infections.

See also Candidiasis; Mycology

G

GALLO, ROBERT C. (1937-)

American virologist

Robert C. Gallo, one of the best-known biomedical researchers in the United States, is considered the co-discoverer, along with **Luc Montagnier** at the Pasteur Institute, of the **Human Immunodeficiency Virus** (**HIV**). Gallo established that the virus causes acquired **immunodeficiency** syndrome (**AIDS**), something that Montagnier had not been able to do, and he developed the blood test for HIV, which remains a central tool in efforts to control the disease. Gallo also discovered the **human T-cell leukemia virus** (**HTLV**) and the human T-cell growth factor interleukin–2.

Gallo's initial work on the isolation and identification of the AIDS virus has been the subject of a number of allegations, resulting in a lengthy investigation and official charges of scientific misconduct which were overturned on appeal. Although he has now been exonerated, the ferocity of the controversy has tended to obscure the importance of his contributions both to AIDS research and biomedical research in general. As Malcolm Gladwell observed in 1990 in the *Washington Post*: "Gallo is easily one of the country's most famous scientists, frequently mentioned as a Nobel Prize contender, and a man whose research publications were cited by other researchers publishing their own work during the last decade more often than those of any other scientist in the world."

Gallo was born in Waterbury, Connecticut, on March 23, 1937, to Francis Anton and Louise Mary (Ciancuilli) Gallo. He grew up in the house that his Italian grandparents bought after they came to the United States. His father worked long hours at the welding company which he owned. The dominant memory of Gallo's youth was of the illness and death of his only sibling, Judy, from childhood leukemia. The disease brought Gallo into contact with the nonfamily member who most influenced his life, Dr. Marcus Cox, the pathologist who diagnosed her disease in 1948. During his senior year in high school, an injury kept Gallo off the high school basketball team and forced him to think about his future. He began to spend time with Cox, visiting him at the hospital, even assisting in postmortem examinations. When Gallo entered college, he knew he wanted a career in biomedical research.

Gallo attended Providence College, where he majored in biology, graduating with a bachelor's degree in 1959. He continued at Jefferson Medical College in Philadelphia, where he got an introduction to medical research. In 1961, he worked as a summer research fellow in Alan Erslev's laboratory at Jefferson. His work studying the pathology of oxygen deprivation in coal miners led to his first scientific publication in 1962, while he was still a medical student.

In 1961, Gallo married Mary Jane Hayes, whom he met while in Providence College. Together they had two children. Gallo graduated from medical school in 1963; on the advice of Erslev, he went to the University of Chicago because it had a reputation as a major center for blood-cell biology, Gallo's research interest. From 1963 to 1965, he did research on the biosynthesis of hemoglobin, the protein that carries oxygen in the blood.

In 1965, Gallo was appointed to the position of clinical associate at the National Institutes of Health (NIH) in Bethesda, Maryland. He spent much of his first year at NIH caring for cancer patients. Despite the challenges, he observed some early successes at treating cancer patients with **chemotherapy**. Children were being cured of the very form of childhood leukemia that killed his sister almost twenty years before. In 1966, Gallo was appointed to his first full-time research position, as an associate of Seymour Perry, who was head of the medicine department. Perry was studying how white blood cells grow in various forms of leukemia. In his laboratory, Gallo studied the **enzymes** involved in the synthesis of the components of **DNA** (**deoxyribonucleic acid**), the carrier of genetic information.

The expansion of the NIH and the passage of the National Cancer Act in 1971 led to the creation of the Laboratory of Tumor Cell Biology at the National Cancer Institute (NCI), a part of the NIH. Gallo was appointed head of the new laboratory. He had become intrigued with the pos-

sibility that certain kinds of cancer had viral origins, and he set up his new laboratory to study human **retroviruses**. Retroviruses are types of viruses that possess the ability to penetrate other cells and splice their own genetic material into the genes of their hosts, eventually taking over all of their reproductive functions. At the time Gallo began his work, retroviruses had been found in animals; the question was whether they existed in humans. His research involved efforts to isolate a virus from victims of certain kinds of leukemia, and he and his colleagues were able to view a retrovirus through electron microscopes. In 1975, Gallo and Robert E. Gallagher announced that they had discovered a human leukemia virus, but other laboratories were unable to replicate their results. Scientists to whom they had sent samples for independent confirmation had found two different retroviruses not from humans, but from animals. The samples had been contaminated by **viruses** from a monkey or a chimp.

Despite the setback, Gallo continued his efforts to isolate a human retrovirus. He turned his attention to T-cells, white blood cells which are an important part of the body's **immune system**, and developed a substance called T-cell growth factor (later called interleukin–2), which would sustain them outside the human body. The importance of this growth factor was that it enabled Gallo and his team to sustain cancerous T-cells long enough to discover whether a retrovirus existed within them. These techniques allowed Gallo and his team to isolate a previously unknown virus from a leukemia patient. He named the virus human T-cell leukemia virus, or HTLV, and he published this finding in *Science* in 1981. This time his findings were confirmed.

It was Gallo's experience with viral research that made him important in the effort to identify the cause of AIDS, after that disease had first been characterized by doctors in the United States. In further studies of HTLV, Gallo had established that it could be transmitted by breast-feeding, sexual intercourse, and blood transfusions. He also observed that the incidence of cancers caused by this virus was concentrated in Africa and the Caribbean. HTLV had these and other characteristics in common with what was then known about AIDS, and Gallo was one of the first scientists to hypothesize that the disease was caused by a virus. In 1982, the National Cancer Institute formed an AIDS task force with Gallo as its head. In this capacity he made available to the scientific community the research methods he had developed for HTLV, and among those whom he provided with some early technical assistance was Luc Montagnier at the Pasteur Institute in Paris.

Gallo tried throughout 1983 to get the AIDS virus to grow in **culture**, using the same growth factor that had worked in growing HTLV, but he was not successful. Finally, a member of Gallo's group named Mikulas Popovic developed a method to grow the virus in a line of T-cells. The method consisted, in effect, of mixing samples from various patients into a kind of a cocktail, using perhaps ten different strains of the virus at a time, so there was a higher chance that one would survive. This innovation allowed the virus to be studied, and observing the similarities to the retroviruses he had previously discovered, Gallo called it HTLV–3. In 1984, he and his colleagues published their findings in *Science*. Gallo and the other scientists in his laboratory were able to establish that this virus caused AIDS, and they developed a blood test for the virus.

Almost a year before Gallo announced his findings, Montagnier at the Pasteur Institute had identified a virus he called LAV, though he was not able to prove that it caused AIDS. The two laboratories were cooperating with each other in the race to find the cause of AIDS and several samples of this virus had been sent to Gallo at the National Cancer Institute. The controversy which would embroil the American scientist's career for almost the next decade began when the United States government denied the French scientists a patent for the AIDS test and awarded one to his team instead. The Pasteur Institute believed their contribution was not recognized in this decision, and they challenged it in court. Gallo did not deny that they had preceded him in isolating the virus, but he argued that it was proof of the causal relationship and the development of the blood test which were most important, and he maintained that these advances had been accomplished using a virus which had been independently isolated in his laboratory.

This first stage of the controversy ended in a legal settlement that was highly unusual for the scientific community: Gallo and Montagnier agreed out of court to share equal credit for their discovery. This settlement followed a review of records from Gallo's laboratory and rested on the assumption that the virus Gallo had discovered was different from the one Montagnier had sent him. An international committee renamed the virus HIV, and in what Specter calls "the first such negotiated history of a scientific enterprise ever published," the American and French groups published an agreement about their contributions in *Nature* in 1987. In 1988, Gallo and Montagnier jointly related the story of the discoveries in *Scientific American*.

Questions about the isolation of the AIDS virus were revived in 1989 by a long article in the *Chicago Tribune*. The journalist, a Pulitzer Prize winner named John Crewdson, had spent three years investigating Gallo's laboratory, making over one hundred requests under the Freedom of Information Act. He directly questioned Gallo's integrity and implied he had stolen Montagnier's virus. The controversy intensified when it was established that the LAV virus which the French had isolated and the HTLV–3 virus were virtually identical. The genetic sequencing in the two were in fact so close that some believed they actually came from the same AIDS patient, and Gallo was accused of simply renaming the virus Montagnier had sent him. Gallo's claim to have independently isolated the virus was further damaged when it was discovered that in the 1984 *Science* article announcing his discovery of HTLV–3 he had accidently published a photograph of Montagnier's virus.

In 1990, pressure from a congressional committee forced the NIH to undertake an investigation. The NIH investigation found Popovic guilty of scientific misconduct but Gallo guilty only of misjudgment. A committee of scientists that oversaw the investigation was strongly critical of these conclusions, and the group expressed concern that Popovic had been assigned more than a fair share of the blame. In June 1992, the NIH investigation was superseded by the Office of Research Integrity (ORI) at the Department of Health and

Human Services, and in December of that year, ORI found both Gallo and Popovic guilty of scientific misconduct. Based largely on a single sentence in the 1984 *Science* article that described the isolation of the virus, the ORI report found Gallo guilty of misconduct for "falsely reporting that LAV had not been transmitted to a permanently growing cell line." This decision renewed the legal threat from the Pasteur Institute, whose lawyers moved to claim all the back royalties from the AIDS blood test, which then amounted to approximately $20 million.

Gallo strongly objected to the findings of the ORI, pointing to the fact that the finding of misconduct turned on a single sentence in a single paper. Other scientists objected to the panel's priorities, arguing that the charge of misconduct concerned a misrepresentation of a relatively minor issue which did not negate the scientific validity of Gallo's conclusions. Lawyers representing both Gallo and Popovic brought their cases before an appeals board at the Department of Health and Human Services. Popovic's case was heard first, and in December 1993, the board announced that he had been cleared of all charges. As quoted in *Time,* the panel declared: "One might anticipate... after all the sound and fury, there would be at least a residue of palpable wrongdoing. This is not the case." The ORI immediately withdrew all charges against Gallo for lack of proof.

According to *Time,* in December 1993, Gallo considered himself "completely vindicated" of all the allegations that had been made against him. He has established that before 1984 his laboratory had succeeded in isolating other strains of the virus that were not similar to LAV. Many scientists now argue that the problem was simply one of **contamination**, a mistake which may have been a consequence of the intense pressure for results in many laboratories during the early years of the AIDS epidemic. It has been hypothesized that the LAV sample from the Pasteur Institute contaminated the mixture of AIDS viruses that Popovic concocted to find one strain that would survive in culture; it is believed that this strain was strong enough to survive and be identified by Gallo and Popovic for a second time.

In 1990, when the controversy was still at its height, Gallo published a book about his career called *Virus Hunting,* which seemed intended to refute the charges against him, particularly the *Tribune* article by Crewdson. Gallo made many of the claims that were later supported by the appeals board, and in the *New York Times Book Review,* Natalie Angier called him "a formidable gladiator who firmly believes in the importance of his scientific contributions." Angier wrote of the book: "His description of the key experiments in 1983 and 1984 that led to the final isolation of the AIDS virus are intelligent and persuasive, particularly to a reader who was heard the other side of the story."

The many allegations and the long series of investigations have distracted many people from the accomplishments of a man whose name appears on hundreds of scientific papers and who has won most major awards in biomedical research except the Nobel Prize. Gallo received the coveted Albert Lasker Award twice, once in 1982 for his work on the viral origins of cancer, and again in 1986 for his research on AIDS. He has also been awarded the American Cancer Society Medal of Honor in 1983, the Lucy Wortham Prize from the Society for Surgical Oncology in 1984, the Armand Hammer Cancer Research Award in 1985, and the Gairdner Foundation International Award for Biomedical Research in 1987. He has received eleven honorary degrees.

See also AIDS, recent advances in research and treatment; Antibody and antigen; Antibody formation and kinetics; Antibody-antigen, biochemical and molecular reactions; Viruses and responses to viral infection

GAS VACUOLES AND GAS VESICLES

Gas vacuoles are aggregates of hollow cylindrical structures called gas vesicles. They are located inside some **bacteria**. A membrane that is permeable to gas bound each gas vesicle. The inflation and deflation of the vesicles provides buoyancy, allowing the bacterium to float at a desired depth in the water.

Bacteria that are known as cyanobacteria contain gas vacuoles. Cyanobacteria, which used to be called **blue-green algae**, live in water and manufacture their own food from the photosynthetic energy of sunlight. Studies have demonstrated that the inflation and deflation of the gas vesicles is coordinated with the light. The buoyancy provided by the gas vacuoles enables the bacteria to float near the surface during the day to take advantage of the presence of sunlight for the manufacture of food, and to sink deeper at night to harvest nutrients that have sunk down into the water.

Gas vesicles are also found in some archae, bacteria that are thought to have branched off from a common ancestor of **eukaryotes** and prokaryotes at a very early stage in **evolution**. For example, the gas vesicles in the bacterium *Halobacterium NRC-1* allow the bacteria to float in their extremely salt water environments (the bacteria are described as halophilic, or "salt loving." The detailed genetic analysis that has been done with this bacterium indicates that at least 13 to 14 genes are involved in production of the two gas vesicle structural proteins and other, perhaps regulatory, proteins. For example, some proteins may sense the environment and act to trigger synthesis of the vesicles. Vesicle synthesis is known to be triggered by low oxygen concentrations.

The gas vesicles tend to be approximately 75 nanometers in diameter. Their length is variable, ranging from 200 to 1000 nanometers, depending on the species of bacteria. The vesicles are constructed of a single small protein. In at least some vesicles these proteins are linked together by another protein. The interior of the protein shell is very **hydrophobic** (water-hating), so that water is excluded from the inside of the vesicles. Yet it is still unclear how the regular arrangement of proteins produces a shell that is permeable to gas. Presumably there must be enough space in between the protein subunits to permit the passage of air.

See also Blue-green algae; Photosynthetic microorganisms

GASTROENTERITIS

Gastroenteritis is an **inflammation** of the stomach and the intestines. More commonly, gastroenteritis is called the stomach flu.

The symptoms of gastroenteritis always include diarrhea. Fever, and vomiting can also be present. Typically the symptoms associated with a bout of gastroenteritis typically last only several days and are self-limiting. But sometimes the malady can be more extended.

The diarrhea in gastroenteritis is very loose, even watery. Also, bowel movements are frequent, occurring even several times an hour as the body attempts to expel the offending microorganism. This large loss of fluid creates the potential for dehydration. Usually dehydration is not an issue in an adult, unless the person is incapable of caring for themselves and has no other caregiver. Dehydration is an important issue in children. If a child is hospitalized because of diarrhea, it is usually because of complications arising from dehydration, rather than from the actual stomach and intestinal infection.

The other symptoms of gastroenteritis are especially complicating in children. Vomiting makes it difficult to administer drugs to combat a **bacterial infection**. Also, the loss of stomach contents can exacerbate dehydration.

Gastroenteritis-induced diarrhea is one of the major causes of death in infants around the world. In Asia, Africa, and Latin America millions of deaths in the newborn to four years age group occurs every year.

Gastroenteritis can be caused by **viruses** and **bacteria**. Viruses are the more common cause. Many There **types of viruses** can cause gastroenteritis. These include rotaviruses, enteroviruses, **adenoviruses**, caliciviruses, astroviruses, Norwalk virus and a group of Norwalk-like viruses. Of these, rotavirus infections are the most common.

Viral gastroenteritis tends to appear quickly, within three days of ingestion of the virus, and diminishes within a week. Those whose **immune system** is compromised may experience symptoms for a longer period of time.

Rotavirus is a virus that contains **ribonucleic acid** as the genetic material. The genetic material is enclosed within a double shell of protein. The virus is a member of the Reoviridae family of viruses. There are three main groups of rotavirus with respect to the antibodies that are produced against them. These types are called groups A, B, and C. Group A rotavirus is the cause of more than three million cases of gastroenteritis in the United States every year. The group B rotavirus causes diarrhea in adults, and has been the cause a several major outbreaks of severe diarrhea in China. Finally, the group C rotavirus can cause diarrhea in both children and adults, but is encountered much less frequently than groups A and B.

Rotavirus gastroenteritis is very contagious, spreading from person to person in a fecal to oral route. Not surprisingly, the virus is frequently encountered in day care facilities, where the care of the soiled diapers of infants occurs regularly. Improper **hygiene**, especially hand washing, contributes directly to the spread of the virus. Infected individuals can shed large numbers of virus in their diarrhea. Infection can also be spread by the **contamination** of eating utensils. Food can become contaminated if the food handler has not properly washed their hands after using the bathroom. Shellfish can also be a source of the virus. Because shellfish feed by filtering water through a special filter feeding apparatus, virus in the water can become trapped and concentrated inside the shellfish. Eating the shellfish, especially raw, spreads the virus.

Gastroenteritis due to the Norwalk virus tends to be more common in adults. However, more advanced immunological methods of detection have detected **antibody** to the virus in many children. Thus, children may be infected by the virus but show no symptoms. Infection in the adult years produces gastroenteritis, for reasons that are as yet unknown. Discovering the nature of the asymptomatic response of children could led to a therapeutic strategy for the adult infection.

Bacteria also cause gastroenteritis. The bacteria of concern include certain strains of ***Escherichia coli, Salmonella, Shigella***, and *Vibrio cholerae*. In developed countries, where sanitary conditions and water treatment are established, bacterial gastroenteritis is infrequent. But the bacterial form remains problematic in the under-developed world, where water is more vulnerable to contamination. Bacterial gastroenteritis can also be caused by the ingestion of contaminated food. For example the presence of *Salmonella* in potato salad that has been improperly stored or of ***E.coli O157:H7*** in undercooked meat can cause the malady.

The protozoan *Cryptosporidium parvum* also causes gastroenteritis following the ingestion of contaminated water.

The bacterial and protozoan cases of gastroenteritis account for well below half of the reported cases. The majority of cases are of viral origin.

In the treatment of gastroenteritis it is important to establish whether the source of the condition is bacterial, viral, protozoan or another and non-biological factor. Intolerance to the digestion of the lactose constituent of milk can also cause gastroenteritis, for example. The need to establish the origin of the malady is important, since bacterial infections will respond to the administration of **antibiotics** while viral infections will not. Furthermore, the use of antibiotics in a viral infection can actually exacerbate the diarrhea.

In August 1998, a **vaccine** for rotavirus gastroenteritis was licensed for sale in the United States. From September 1998 until July 1999, 15 cases of intussusception (a condition where a segment of bowel folds inside an adjacent segment, causing an obstruction) were reported among infants who received the vaccine. Subsequently, the vaccine was withdrawn from the market. No other vaccine has as yet been licensed for use.

See also Enterobacterial infections; Transmission of pathogens

GENE

A gene is the fundamental physical and functional unit of heredity. Whether in a microorganism or in a human cell, a

gene is an individual element of an organism's genome and determines a trait or characteristic by regulating biochemical structure or metabolic process.

Genes are segments of nucleic acid, consisting of a specific sequence and number of the chemical units of nucleic acids, the nucleotides. In most organisms, the nucleic acid is **DNA** (**deoxyribonucleic acid**), although in **retroviruses**, the genetic material is composed of **ribonucleic acid** (**RNA**). Some genes in a cell are active more or less all the time, which means they are continuously transcribed and provide a constant supply of their protein product. These "housekeeping" genes are always needed for basic cellular reactions. Others may be rendered active or inactive depending on the needs and functions of the organism under particular conditions. The signal that masks or unmasks a gene can come from outside the cell, for example, from a steroid hormone or a nutrient, or it can come from within the cell itself because of the activity of other genes. In both cases, regulatory substances can bind to the specific DNA sequences of the target genes to control the synthesis of transcripts.

In a paper published in 1865, Gregor Mendel (1823–1884), advanced a theory of inheritance dependent on material elements that segregate independently from each other in sex cells. Before Mendel's findings, inherited traits were thought to be passed on through a blending of the mother and father's characteristics, much like a blending of two liquids. The term "gene" was coined later by the Danish botanist Wilhelm Johannsen (1857–1927), to replace the variety of terms used up until then to describe hereditary factors. His definition of the gene led him to distinguish between **genotype** (an organism's genetic makeup) and **phenotype** (an organism's appearance). Before the chemical and physical nature of genes were discovered they were defined on the basis of phenotypic expression and algebraic symbols were used to record their distribution and segregation. Because sexually reproducing, eukaryotic organisms possess two copies of an inherited factor (or gene), one acquired from each parent, the genotype of an individual for a particular trait is expressed by a pair of letters or symbols. Each of the alternative forms of a gene is also known as alleles. Dominant and recessive alleles are denoted by the use of higher and lower case letters. It can be predicted mathematically, for example, that a single allele pair will always segregate to give a genotype ratio 1AA:2Aa:1aa, and the phenotype ratio 2A:1aa (where A represents both AA and Aa since these cannot be distinguished phenotypically if dominance is complete).

The molecular structure and activity of genes can be modified by **mutations** and the smallest mutational unit is now known to be a single pair of nucleotides, also known as a muton. To indicate that a gene is functionally normal it is assigned a plus (+) sign, whereas a damaged or mutated gene is indicated by a minus (–) sign. A wild-type ***Escherichia coli*** able to synthesize its own arginine would thus, be symbolized as arg^+ and strains that have lost this ability by mutation of one of the genes for arginine utilization would be arg^-. Such strains, known as arginine auxotrophs, would not be able to grow without a supplement of arginine. At this level of definition, the plus or minus actually refer to an **operon** rather than a single gene, and finer genetic analysis can be used to reveal the exact location of the mutated gene.

The use of mutations in studying genes is well illustrated in a traditional genetic test called the "*cis–trans* test" which also gave the gene the alternative name, cistron. This is a complementation test that can be used to determine whether two different mutations (m^1 and m^2) occur in the same functional unit, i.e., within the same gene or cistron. It demonstrates well how genes can be defined phenomenologically and has been performed successfully in **microorganisms** such as yeasts. It works on the principle that pairs of homologous **chromosomes** containing similar genes can **complement** their action. Two types of heterozygotes of the test organism are prepared. Heterozygotes are organisms having different alleles in the two homologous chromosomes each of which was inherited from one parent. One heterozygote contains the mutations under investigation within the same chromosome, that is in the *cis*— configuration, which is symbolically designated $++/m^1m^2$ (m^1 and m^2 are the two mutations under investigation and the symbol "+" indicates the same position on the homologous chromosome in the unmutated, wild type state). The second mutant is constructed to contain the mutations in such a way that one appears on each of the homologous chromosomes. This is called the *trans*— configuration and is designated, for example, by $^2+/+m^1$. If two recessive mutations are present in the same cistron, the heterozygous *trans*— configuration displays the mutant phenotype, whereas the *cis*— configuration displays the normal, wild type, phenotype. This is because in the *cis*— configuration, there is one completely functional, unmutated, cistron (++) within the system which masks the two mutations on the other chromosome and allows for the expression of the wild type phenotype. If one or both mutations are dominant, and the *cis*– and *trans*– heterozygotes are phenotypically different, then both mutations must be present in the same cistron. Conversely, if the *cis*– and *trans*– heterozygotes are phenotypically identical, this is taken as evidence that the mutations are present in different cistrons.

In 1910, the American geneticist Thomas Hunt Morgan (1866–1945) began to uncover the relationship between genes and chromosomes. He discovered that genes were located on chromosomes and that they were arranged linearly and associated in linkage groups, all the genes on one chromosome being linked. For example the genes on the X and Y chromosomes are said to be sex-linked because the X and Y chromosomes determine the sex of the organisms, in humans X determining femaleness and Y determining maleness. Nonhomologous chromosomes possess different linkage groups, whereas homologous chromosomes have identical linkage groups in identical sequences. The distance between two genes of the same linkage group is the sum of the distances between all the intervening genes and a schematic representation of the linear arrangement of linked genes, with their relative distances of separation, is known as a genetic map. In the construction of such maps the frequency of **recombination** during crossing over is used as an index of the distance between two linked genes.

Advances in **molecular genetics** have allowed analysis of the structure and **biochemistry** of genes in detail. They are

no longer the nebulous units described by Mendel purely in terms of their visible expression (phenotypic expression). It is now possible to understand their molecular structure and function in considerable detail. The biological role of genes is to carry, encode, or control information on the composition of proteins. The proteins, together with their timing of expression and amount of production are possibly the most important determinants of the structure and physiology of organisms. Each structural gene is responsible for one specific protein or part of a protein and codes for a single polypeptide chain via messenger RNA (mRNA). Some genes code specifically for transfer RNA (tRNA) or ribosomal RNA (rRNA) and some are merely sequences, which are recognized by regulatory proteins. The latter are termed regulator genes. In higher organisms, or **eukaryotes**, genes are organized in such a way that at one end, there is a region to which various regulatory proteins can bind, for example RNA polymerase during **transcription**, and at the opposite end, there are sequences encoding the termination of transcription. In between lies the protein encoding sequence. In the genes of many eukaryotes this sequence may be interrupted by intervening non-coding sequence segments called introns, which can range in number from one to many. Transcription of eukaryotic DNA produces pre–mRNA containing complementary sequences of both introns and the information carrying sections of the gene called exons. The pre–mRNA then undergoes post–transcriptional modification or processing in which the introns are excised and exons are spliced together, leaving the complete coding transcript of connected exons ready to code directly for the protein. When the central dogma of genetics was first established, a "one gene–one enzyme" hypothesis was proposed, but today it is more accurate to restate this as a one to one correspondence between a gene and the polypeptide for which it codes. This is because a number of proteins are now known to be constituted of multiple polypeptide subunits coded for by different genes.

See also Bacterial artificial chromosome (BAC); Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); Evolution and evolutionary mechanisms; Gene amplification; Genetic code; Genetic mapping; Genotype and phenotype; Immunogenetics; Microbial genetics; Molecular biology, central dogma of; Molecular biology and molecular genetics

GENE CHIPS

• *see* DNA CHIPS AND MICROARRAYS

GENETIC CAUSES OF IMMUNODEFICIENCY

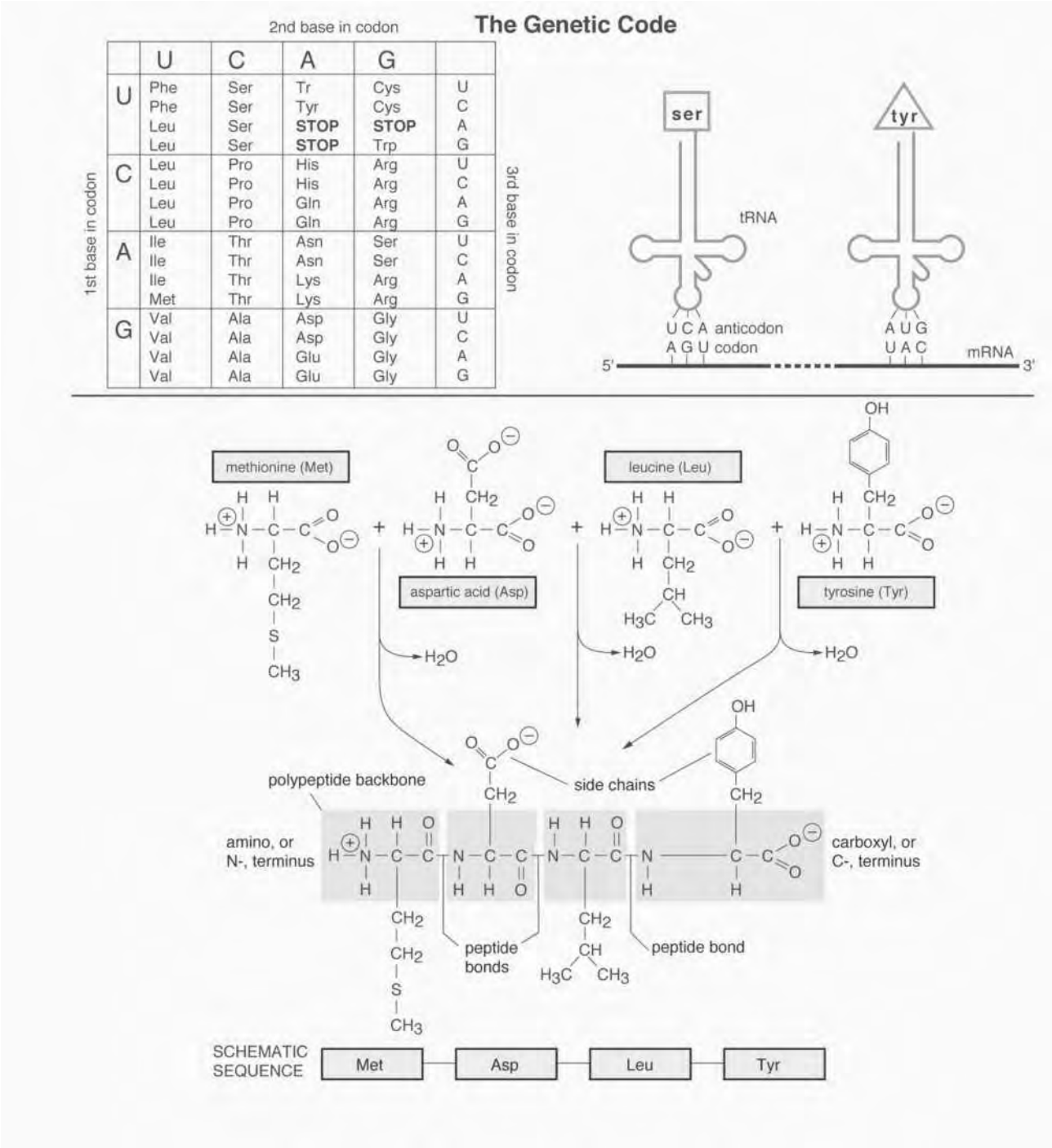
• *see* IMMUNODEFICIENCY DISEASE SYNDROMES

GENETIC CODE

The genetic code is the set of correspondences between the nucleotide sequences of nucleic acids such as **DNA** (**deoxyribonucleic acid**), and the amino acid sequences of polypeptides. These correspondences enable the information encoded in the chemical components of the DNA to be transferred to the **ribonucleic acid** messenger (mRNA), and then to be used to establish the correct sequence of amino acids in the polypeptide. The elements of the encoding system, the nucleotides, differ by only four different bases. These are known as adenine (A), guanine, (G), thymine (T) and cytosine (C), in DNA or uracil (U) in **RNA**. Thus, RNA contains U in the place of C and the nucleotide sequence of DNA acts as a template for the synthesis of a complementary sequence of RNA, a process known as **transcription**. For historical reasons, the term genetic code in fact refers specifically to the sequence of nucleotides in mRNA, although today it is sometimes used interchangeably with the coded information in DNA.

Proteins found in nature consist of 20 naturally occurring amino acids. One important question is, how can four nucleotides code for 20 amino acids? This question was raised by scientists in the 1950s soon after the discovery that the DNA comprised the hereditary material of living organisms. It was reasoned that if a single nucleotide coded for one amino acid, then only four amino acids could be provided for. Alternatively, if two nucleotides specified one amino acid, then there could be a maximum number of 16 (4^2) possible arrangements. If, however, three nucleotides coded for one amino acid, then there would be 64 (4^3) possible permutations, more than enough to account for all the 20 naturally occurring amino acids. The latter suggestion was proposed by the Russian born physicist, George Gamow (1904–1968) and was later proved correct. It is now well known that every amino acid is coded by at least one nucleotide triplet or codon, and that some triplet combinations function as instructions for the termination or initiation of **translation**. Three combinations in tRNA, UAA, UGA and UAG, are termination codons, while AUG is a translation start codon.

The genetic code was solved between 1961 and 1963. The American scientist Marshall Nirenberg (1927–), working with his colleague Heinrich Matthaei, made the first breakthrough when they discovered how to make synthetic mRNA. They found that if the nucleotides of RNA carrying the four bases A, G, C and U, were mixed in the presence of the enzyme polynucleotide phosphorylase, a single stranded RNA was formed in the reaction, with the nucleotides being incorporated at random. This offered the possibility of creating specific mRNA sequences and then seeing which amino acids they would specify. The first synthetic mRNA polymer obtained contained only uracil (U) and when mixed *in vitro* with the protein synthesizing machinery of ***Escherichia coli*** it produced a polyphenylalanine—a string of phenylalanine. From this it was concluded that the triplet UUU coded for phenylalanine. Similarly, a pure cytosine (C) RNA polymer produced only the amino acid proline so the corresponding codon for cytosine had to be CCC. This type of analysis was refined when nucleotides were mixed in different proportions in the synthetic mRNA and a statistical analysis was used to determine the amino acids produced. It was quickly found that a particular amino acid could be specified by more than one codon. Thus, the amino acid serine could be produced from any one of the combinations UCU, UCC, UCA, or UCG. In this way the genetic code is said to be degenerate, meaning that each of the 64 possible



Genetic code related to models of amino acids inserting into a protein.

triplets have some meaning within the code and that several codons may encode a single amino acid.

This work confirmed the ideas of the British scientists **Francis Crick** (1916–) and **Sidney Brenner** (1927–). Brenner and Crick were working with **mutations** in the bacterial virus bactriophage T4 and found that the deletion of a single nucleotide could abolish the function of a specific **gene**. However, a second mutation in which a nucleotide was inserted at a different, but nearby position restored the function of that gene. These two mutations are said to be suppressors of each other, meaning that they cancel each other's mutant properties. It was concluded from this that the genetic code was

read in a sequential manner starting from a fixed point in the gene. The insertion or deletion of a nucleotide shifted the reading frame in which succeeding nucleotides were read as codons, and was thus termed a frameshift mutation. It was also found that whereas two closely spaced deletions, or two closely spaced insertions, could not suppress each other, three closely spaced deletions or insertions could do so. Consequently, these observations established the triplet nature of the genetic code. The reading frame of a sequence is the way in which the sequence is divided into the triplets and is determined by the precise point at which translation is initiated. For example, the sequence CATCATCAT can be read CAT CAT CAT or C ATC ATC AT or CA TCA TCA T in the three possible reading frames. Sometimes, as in particular bacterial **viruses**, genes have been found that are contained within other genes. These are translated in different reading frames so the amino acid sequences of the proteins encoded by them are different. Such economy of genetic material is, however, quite rare

The same genetic code appears to operate in all living things, but exceptions to this universality are known. In human mitochondrial mRNA, AGA and AGG are termination or stop codons. Other differences also exist in the correspondences between certain codon sequences and amino acids. In ciliates, there are also unusual features in that UAA and UAG code for glutamine (CAA and CAG in other **eukaryotes**) and the only termination codon appears to be UGA.

See also Bacteriophage and bacteriophage typing; Gene amplification; Genetic identification of microorganisms; Genetic mapping; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Genotype and phenotype; Immunogenetics

GENETIC IDENTIFICATION OF MICROORGANISMS

The genetic identification of **microorganisms** utilizes molecular technologies to evaluate specific regions of the genome and uniquely determine to which genus, species, or strain a microorganism belongs. This work grew out of the similar, highly successful applications in human identification using the same basic techniques. Thus, the genetic identification of microorganisms has also been referred to a microbial fingerprinting.

Genetic identification of microorganisms is basically a comparison study. To identify an unknown organism, appropriate sequences from the unknown are compared to documented sequences from known organisms. Homology between the sequences results in a positive test. An exact match will occur when the two organisms are the same. Related individuals have genetic material that is identical for some regions and dissimilar for others. Unrelated individuals will have significant differences in the sequences being evaluated. Developing a database of key sequences that are unique to and characteristic of a series of known organisms facilitates this type of analysis. The sequences utilized fall into two different categories, 1) fragments derived from the transcriptionally active, coding regions of the genome, and, 2) fragments present in inactive, noncoding regions. Of the two, the noncoding genomic material is more susceptible to mutation and will therefore show a higher degree of variability.

Depending on the level of specificity required, an assay can provide information on the genus, species, and/or strain of a microorganism. The most basic type of identification is classification to a genus. Although this general identification does not discriminate between the related species that comprise the genus, it can be useful in a variety of situations. For example, if a person is thought to have **tuberculosis**, a test to determine if *Mycobacterium* cells (the genus that includes the tuberculosis causing organism) are present in a sputum sample will most likely confirm the diagnosis. However, if there are several species within a genus that cause similar diseases but that respond to entirely different drugs, it would then be critical to know exactly which species is present for proper treatment. A more specific test using genomic sequences unique to each species would be needed for this type of discrimination. In some instances, it is important to take the analysis one step further to detect genetically distinct subspecies or strains. Variant strains usually arise as a result of physical separation and **evolution** of the genome. If one homogeneous sample of cells is split and sent to two different locations, over time, changes (**mutations**) may occur that will distinguish the two populations as unique entities. The importance of this issue can be appreciated when considering tuberculosis. Since the late 1980s, there has been a resurgence of this disease accompanied by the appearance of several new strains with antimicrobial resistance. The use of genetic identification for rapid determination of which strain is present has been essential to protect health care workers and provide appropriate therapy for affected individuals.

The tools used for genetic studies include standard molecular technologies. Total sequencing of an organism's genome is one approach, but this method is time consuming and expensive. Southern blot analysis can be used, but has been replaced by newer technologies in most laboratories. Solution-phase hybridization using **DNA** probes has proven effective for many organisms. In this procedure, probes labeled with a reporter molecule are combined with cells in solution and upon hybridization with target cells, a chemiluminescent signal that can be quantitated by a luminometer is emitted. A variation of this scheme is to capture the target cells by hybridization to a probe followed by a second hybridization that results in precipitation of the cells for quantitation. These assays are rapid, relatively inexpensive and highly sensitive. However, they require the presence of a relatively large number of organisms to be effective. Amplification tech nologies such as **PCR** (**polymerase chain reaction**) and LCR (ligase change reaction) allow detection of very low concentrations of organisms from cultures or patient specimens such as blood or body tissues. Primers are designed to selectively amplify genomic sequences unique to each species, and, by screening unknowns for the presence or absence these regions, the unknown is identified. Multiplex PCR has made it possible to discriminate between a number of different species in a

Technician making a genetic marker.

single amplification reaction. For **viruses** with a **RNA** genome, RT-PCR (reverse transcriptase PCR) is widely utilized for identification and quantitation.

The **anthrax** outbreak in the Unites States in the fall of 2001 illustrated the significance of these technologies. Because an anthrax infection can mimic **cold** or flu symptoms, the earliest victims did not realize they were harboring a deadly bacterium. After confirmation that anthrax was the causative agent in the first death, genetic technologies were utilized to confirm the presence of anthrax in other locations and for other potential victims. Results were available more rapidly than would have been possible using standard microbiological methodology and appropriate treatment regimens could be established immediately. Furthermore, unaffected individuals are quickly informed of their status, alleviating unnecessary anxiety.

The second stage of the investigation was to locate the origin of the anthrax cells. The evidence indicated that this event was not a random, natural phenomenon, and that an individual or individuals had most likely dispersed the cells as an act of **bioterrorism**. In response to this threat, government agencies collected samples from all sites for analysis. A key element in the search was the genetic identification of the cells found in patients and mail from Florida, New York, and Washington, D.C. The PCR studies clearly showed that all samples were derived from the same strain of anthrax, known as the "Ames strain" since the cell line was established in Iowa. Although this strain has been distributed to many different research laboratories around the world, careful analysis revealed minor changes in the genome that allowed investigators to narrow the search to about fifteen United States laboratories. Total genome sequencing of these fifteen strains and a one-to-one base comparison with the lethal anthrax genome may detect further variation that will allow a unique identification to be made.

The advent of molecular technologies and the application of genetic identification in clinical and forensic microbiology have greatly improved the capability of laboratories to detect and specifically identify an organism quickly and accurately.

See also Anthrax, terrorist use of as a biological weapon; Culture; Microbial genetics

GENETIC MAPPING

The aim of genetic mapping is to determine the linear sequence of genes in genetic material. The mapping can be performed at several levels of detail (resolution) that fall into two broad types: traditional genetic or linkage mapping and, more detailed, physical mapping.

Linkage mapping shows the relative rather than absolute positions of genes along a chromosome and is a technique that has been used since the early 1900s. Early geneticists determined that genes were found on **chromosomes**. They also reasoned that because the various forms of genes, or alleles, could be precisely exchanged during meiosis through crossovers between homologous chromosomes, the genes for specific characteristics must lie at precise points along each chromosome. It followed that the mapping of chromosomes could, therefore, be made from the observation of crossovers. Between 1912 and 1915, the American scientist Thomas Hunt Morgan (1866–1945) hypothesized that if genes were arranged linearly along chromosomes, then those genes lying closer together would be separated by crossovers less often than those lying further apart. Genes lying closer together would thus have a greater probability of being passed along as a unit. It follows that the percentage of crossovers would be proportional to the distance between two genes on a chromosome. The percentage crossover can be expressed as the number of crossovers between two genes in meiosis. One genetic map unit (m.u.) is defined as the distance between **gene** pairs for which one product out of 100 is recombinant (a product of crossover). The recombinant frequency (R.F.) of 0.01 (1%) is defined as 1 m.u. and a map unit is sometimes referred to as a centimorgan (cM) in honor of Thomas Hunt Morgan.

As an example of how linkage mapping might work, suppose two characteristics, A and B, show 26% crossover. Assign 26 crossover units to the distance between these two

genes. If a characteristic C turns out in breeding experiments to have 9% crossover with B and 17% crossover with A, it would then be located between A and B at a point 9 units from B and 17 units from A. Compiling the information from many such breeding experiments creates a chromosome map that indicates the relative positions of the genes that code for certain characteristics. Accordingly, the further apart any two genes are on the same chromosome, the greater the incidence of crossing over between them.

A linkage map is limited because **recombination** frequencies can be distorted relative to the physical distance between sites. As a result, the linkage map is not always the best possible representation of genetic material.

While linkage maps only indicate relative positions of genes, physical maps are more accurate and aim to show the actual number of nucleotides between each gene. Restriction maps are constructed by cleaving **DNA** into fragments with **restriction enzymes**. These **enzymes** recognize specific short DNA sequences and cut the duplex. The distances between the sites of cleavage are then measured. The positions of the target restriction sites for these enzymes along the chromosome can be used as DNA markers. Restriction sites generally exist in the same positions on homologous chromosomes so the positions of these target sites can be used rather like milestones along a road and can act as reference points for locating significant features in the chromosome.

A map of the positions of restriction sites can be made for a localized region of a chromosome. It is made by comparing the sizes of single enzyme breakages (digests) of the region of interest with double digests of the same region. This means that two different restriction enzymes are applied, one to each of two separate chromosome extracts of the region of interest, and subsequently the two enzymes are used together in a third digestion with the chromosome extract. The chromosome fragments resulting from the three digestions are then subjected to a biochemical procedure known as gel **electrophoresis**, which separates them and gives an estimation of their size. Comparison of the sizes of the chromosome fragments resulting from single and double restriction enzyme digestions allows for an approximate location of the target restriction sites. Thus, such maps represent linear sequences of restriction sites. As this procedure determines the sizes of digested chromosome fragments, the distances between sites in terms of the length of DNA can be calculated, because the size of a fragment estimated from an electrophoresis experiment is proportional to the number of base pairs in that fragment.

A restriction map does not intrinsically identify sites if genetic interest. For it to be of practical use, **mutations** have to be characterized in terms of their effects upon the restriction sites. In the 1980s, it was shown how restriction fragment length polymorphisms (RFLPs) could be used to map human disease genes. RFLPs are inherited by Mendelian segregation and are distributed in populations as classical examples of common genetic polymorphisms. If such a DNA variant is located close to a defective gene (which can not be tested directly), the DNA variant can be used as a marker to detect the presence of the disease-causing gene. The prenatal examination of DNA for particular enzyme sites associated with certain hereditary diseases has proved to be an important method of diagnosis. Clinically useful polymorphic restriction enzyme sites have been detected within the Beta-like globin gene cluster. For example, the absence of a recognition site for the restriction enzyme HpaI is frequently associated with the allele for sickle-cell anemia, and this association has been useful in prenatal diagnosis of this disease.

The ultimate genetic map is the complete nucleotide sequence of the DNA in the whole chromosome **complement**, or genome, of an organism. Today, several completed genome maps already exist. Simple prokaryotic organisms, e.g., **bacteria**, with their relatively small (one to two million base pairs) chromosomes of one to two million base pairs were the first to be mapped. Later, eukaryotic organisms such as the **yeast**, ***Saccharomyces cerevisiae***, and the nematode worm, *Caenorhabditis elegans*, were mapped. In 2000, the Human Genome Project produced the first draft of the human genome. The project adopted two methods for mapping the 3 billion nucleotides. The earlier approach was a "clone by clone" method. In this, the entire genome was cut into fragments up to several thousand base pairs long, and inserted into synthetic chromosomes known as **bacterial artificial chromosomes (BACs)**. The subsequent mapping step involved positioning the BACs on the genome's chromosomes by looking for distinctive marker sequences called sequence tagged sites (STSs), whose location had already been pinpointed. Clones of the BACs are then broken into smaller fragments in a process known as **shotgun cloning**. Each small fragment was then sequenced and computer algorithms, that recognize matching sequence information from overlapping fragments, were used to reconstruct the complete sequence inserted into each BAC. It was later argued that the first mapping step was unnecessary and that the algorithms used to reassemble the shotgunned DNA fragments could be applied to cloned random fragments taken directly from the whole genome. In this whole genome shotgun strategy, fragments were first assembled by algorithms into larger scaffolds and the correct position of these scaffolds on the genome was worked out by STSs. The latter method speeded up the whole procedure considerably and is currently being used to sequence genomes from other organisms.

See also Cloning, application of cloning to biological problems; Fungal genetics; Gene amplification; Gene; Genetic code; Genetic identification of microorganisms; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Genotype and phenotype; Microbial genetics

GENETIC REGULATION OF EUKARYOTIC CELLS

Although prokaryotes (i.e., non-nucleated unicellular organisms) divide through binary fission, **eukaryotes** undergo a more complex process of cell division because **DNA** is packed in several **chromosomes** located inside a cell **nucleus**. In

eukaryotes, cell division may take two different paths, in accordance with the cell type involved. Mitosis is a cellular division resulting in two identical nuclei is performed by somatic cells. The process of meiosis results in four nuclei, each containing half of the original number of chromosomes. Sex cells or gametes (ovum and spermatozoids) divide by meiosis. Both prokaryotes and eukaryotes undergo a final process, known as cytoplasmatic division, which divides the parental cell into new daughter cells.

The series of stages that a cell undergoes while progressing to division is known as **cell cycle**. Cells undergoing division are also termed competent cells. When a cell is not progressing to mitosis, it remains in phase G0 (G zero). Therefore, the cell cycle is divided into two major phases: interphase and mitosis. Interphase includes the phases (or stages) G1, S and G2, whereas mitosis is subdivided into prophase, metaphase, anaphase and telophase.

The cell cycle starts in G1, with the active synthesis of **RNA** and proteins, which are necessary for young cells to grow and mature. The time G1 lasts, varies greatly among eukaryotic cells of different species and from one tissue to another in the same organism. Tissues that require fast cellular renovation, such as mucosa and endometrial epithelia, have shorter G1 periods than those tissues that do not require frequent renovation or repair, such as muscles or connective tissues.

The cell cycle is highly regulated by several **enzymes**, proteins, and **cytokines** in each of its phases, in order to ensure that the resulting daughter cells receive the appropriate amount of genetic information originally present in the parental cell. In the case of somatic cells, each of the two daughter cells must contain an exact copy of the original genome present in the parental cell. Cell cycle controls also regulate when and to what extent the cells of a given tissue must proliferate, in order to avoid abnormal cell proliferation that could lead to dysplasia or tumor development. Therefore, when one or more of such controls are lost or inhibited, abnormal overgrowth will occur and may lead to impairment of function and disease.

Cells are mainly induced into proliferation by growth factors or hormones that occupy specific receptors on the surface of the cell membrane, and are also known as extra-cellular ligands. Examples of growth factors are as such: epidermal growth factor (EGF), fibroblastic growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), or hormones. PDGF and FGF act by regulating the phase G2 of the cell cycle and during mitosis. After mitosis, they act again stimulating the daughter cells to grow, thus leading them from G0 to G1. Therefore, FGF and PDGF are also termed competence factors, whereas EGF and IGF are termed progression factors, because they keep the process of cellular progression to mitosis going on. Growth factors are also classified (along with other molecules that promote the cell cycle) as pro-mitotic signals. Hormones are also pro-mitotic signals. For example, thyrotrophic hormone, one of the hormones produced by the pituitary gland, induces the proliferation of thyroid gland's cells. Another pituitary hormone, known as growth hormone or somatotrophic hormone (STH), is responsible by body growth during childhood and early adolescence, inducing the lengthening of the long bones and **protein synthesis**. Estrogens are hormones that do not occupy a membrane receptor, but instead, penetrate the cell and the nucleus, binding directly to specific sites in the DNA, thus inducing the cell cycle.

Anti-mitotic signals may have several different origins, such as cell-to-cell adhesion, factors of adhesion to the extracellular matrix, or soluble factor such as TGF beta (tumor growth factor beta), which inhibits abnormal cell proliferation, proteins p53, p16, p21, APC, pRb, etc. These molecules are the products of a class of genes called tumor suppressor genes. **Oncogenes**, until recently also known as proto-oncogenes, synthesize proteins that enhance the stimuli started by growth factors, amplifying the mitotic signal to the nucleus, and/or promoting the accomplishment of a necessary step of the cell cycle. When each phase of the cell cycle is completed, the proteins involved in that phase are degraded, so that once the next phase starts, the cell is unable to go back to the previous one. Next to the end of phase G1, the cycle is paused by tumor suppressor **gene** products, to allow verification and repair of DNA damage. When DNA damage is not repairable, these genes stimulate other intra-cellular pathways that induce the cell into suicide or apoptosis (also known as programmed cell death). To the end of phase G2, before the transition to mitosis, the cycle is paused again for a new verification and decision, either mitosis or apoptosis.

Along each pro-mitotic and anti-mitotic intra-cellular signaling pathway, as well as along the apoptotic pathways, several gene products (**proteins and enzymes**) are involved in an orderly sequence of activation and inactivation, forming complex webs of signal transmission and signal amplification to the nucleus. The general goal of such cascades of signals is to achieve the orderly progression of each phase of the cell cycle.

Interphase is a phase of cell growth and metabolic activity, without cell nuclear division, comprised of several stages or phases. During Gap 1 or G1, the cell resumes protein and RNA synthesis, which was interrupted during mitosis, thus allowing the growth and maturation of young cells to accomplish their physiologic function. Immediately following is a variable length pause for DNA checking and repair before cell cycle transition to phase S during which there is synthesis or semi-conservative replication or synthesis of DNA. During Gap 2 or G2, there is increased RNA and protein synthesis, followed by a second pause for proofreading and eventual repairs in the newly synthesized DNA sequences before transition to mitosis.

At the start of mitosis, the chromosomes are already duplicated, with the sister-chromatids (identical chromosomes) clearly visible under a light **microscope**. Mitosis is subdivided into prophase, metaphase, anaphase, and telophase.

During prophase there is a high condensation of chromatids, with the beginning of nucleolus disorganization and nuclear membrane disintegration, followed by the start of centrioles' migration to opposite cell poles. During metaphase the chromosomes organize at the equator of a spindle apparatus (microtubules), forming a structure termed metaphase plate.

The sister-chromatids are separated and joined to different centromeres, while the microtubules forming the spindle are attached to a region of the centromere termed kinetochore. During anaphase there are spindles, running from each opposite kinetochore, that pull each set of chromosomes to their respective cell poles, thus ensuring that in the following phase each new cell will ultimately receive an equal division of chromosomes. During telophase, kinetochores and spindles disintegrate, the reorganization of nucleus begins, chromatin becomes less condensed, and the nucleus membrane start forming again around each set of chromosomes. The cytoskeleton is reorganized and the somatic cell has now doubled its volume and presents two organized nucleus.

Cytokinesis usually begins during telophase, and is the process of cytoplasmatic division. This process of division varies among species but in somatic cells, it occurs through the equal division of the cytoplasmatic content, with the plasma membrane forming inwardly a deep cleft that ultimately divides the parental cell in two new daughter cells.

The identification and detailed understanding of the many molecules involved in the cell cycle controls and intracellular signal **transduction** is presently under investigation by several research groups around the world. This knowledge is crucial to the development of new anti-cancer drugs as well as to new treatments for other genetic diseases, in which a gene over expression or deregulation may be causing either a chronic or an acute disease, or the impairment of a vital organ function. Scientists predict that the next two decades will be dedicated to the identification of gene products and their respective function in the cellular microenvironment. This new field of research is termed **proteomics**.

See also Cell cycle (eukaryotic), genetic regulation of; Genetic code; Genetic identification of microorganisms; Genetic mapping

GENETIC REGULATION OF PROKARYOTIC CELLS

The ability of a bacterium to regulate the expression of the myriad of genes contained in the chromosome and **plasmids** is essential to the growth and survival of the microorganism. While a bacterium has many genes that code for a variety of proteins, these genes are not all expressed at the same time. Some genes are active all the time, while others are active only at specific times in the growth cycle of the bacterium or in response to a certain environmental condition. The amounts of proteins that are produced are not all the same. Moreover, the triggers that stimulate the expression of one **gene** can be quite different from the triggers for another gene. The ability of a prokaryotic cells (bacterial are the prototypical system) to orchestrate the expression of the repertoire of genes constitutes genetic regulation.

The activity of genes in the manufacture of compounds by the bacterium, such as in the biosynthetic pathways of the microbe, is often under a type of control known as feedback inhibition. In this type of genetic regulation, the object of the regulation is the first enzyme that is unique to the pathway (not to the gene that coeds for the enzyme). In biosynthetic pathways, there are typically a number of compounds that can be formed in the various enzymatic reactions within the pathway. Feedback inhibition occurs when the final product inhibits the first biosynthetic enzyme. Blocking the first enzymatic step prevent the remainder of the **enzymes** from having any material on which to act.

Feedback inhibition is possible because the biosynthetic enzymes have two binding regions. If both sites are occupied by the end product, the three-dimensional structure of the enzyme is changed such that it cannot bind any more of the protein it is supposed to enzymatically alter. But, when the amount of the end product decreases, one of the enzyme's binding sites is no longer occupied, and the enzyme can resume its function. The function of the enzyme can also be affected by modifying the structure of side groups that protrude from the enzyme molecule. This alteration is also reversible, when the concentration of the blocking end product is lowered.

Feedback inhibition is a genetic regulatory mechanism that allows a bacterium to rapidly respond to changes in concentration of a particular compound. The bacterium does not have to manufacture protein, as the molecules already exist and are primed to resume activity once conditions are favorable.

Regulation also operates directly at the level of the genes. This type of genetic regulation is called induction (when the gene is stimulated into action) or repression (when the gene's activity is reversible silenced). Regulating the activity of genes, rather than the activity of the proteins made by the genes can save a bacterium the energy of manufacturing the protein.

Induction and repression depend on the binding of a molecule known as **RNA** polymerase to regions that signal the beginning of a stretch of **DNA** that code for proteins. The three-dimensional shape of the polymerase-binding region influences the binding of the RNA polymerase. The binding of molecules called effectors can in turn influence the shape of this region. If an effector alters the shape of the polymerase-binding region so that the polymerase is able to bind, the effect is called induction. If the effector binding prevents the polymerase from binding, then the effect is known as repression.

Induction and repression tend to cycle back and forth, in response to the level of effector, and so in response to whatever environmental or other condition the particular effector is sensitive to. A visual analogy would the turning on and off of room light under the control of a very sensitive light meter, as clouds obscured the sunlight from one moment to the next.

Another genetic regulatory mechanism that operates only in procaryotes is termed attenuation. Attenuation requires a close coupling between the synthesis of **ribonucleic acid** from a DNA template (**transcription**) and the use of the RNA as another template to manufacture protein (**translation**). These processes are very closely coupled in procaryotes, particularly in the activity of enzymes that participate in the making of amino acids.

In the process of attenuation, a gene that codes for an enzyme required for the synthesis of an amino acid is not active until the level of that amino acid lowers to some threshold level. At this level, the molecules called **ribosomes** physically stall as they move down the beginning of the RNA template that encodes protein. The stalling prevents the formation of a signal that otherwise stops the onward movement of the ribosomes. After the pause, because the stop signal has not formed, the ribosomes resume their movement and the protein is produced. When the level of the critical amino acid is higher, the ribosomes do not stall, encounter a stop signal, and the synthesis of the protein does not occur.

These processes operate simultaneously for many genes in a bacterium. For some of these genes, the controlling factors are independent of one another. But for other proteins, a common factor, such as a sensory protein that can sense changes in the environment and provide a signal to the various regulatory processes, also operates. This genetic regulation pattern is referred to as global regulation. An example of global regulation is a phenomenon called diauxic growth, which is exemplified by the lactose **operon** (also called the lac operon). Diauxic growth allows a bacterium to preferentially utilize one nutrient (such as glucose) when two nutrients (such as glucose and lactose) are present. When the preferred source is exhausted, **metabolism** can switch so as to utilize the second source (lactose). This nutrient preference involves genetic regulation of protein production.

Other genetic regulatory mechanisms operate in response to fluctuations in temperature, **pH**, oxygen level, the attraction or repulsion of a bacterium from a compound (chemotaxis), and the production of a spore.

See also Cell cycle (prokaryotic), genetic regulation of; Microbial genetics

GENETICALLY ENGINEERED VACCINES

see VACCINE

GENOTYPE AND PHENOTYPE

The term genotype describes the actual set (**complement**) of genes carried by an organism. In contrast, **phenotype** refers to the observable expression of characters and traits coded for by those genes. Although phenotypes are based upon the content of the underlying genes comprising the genotype, the expression of those genes in observable traits (phenotypic expression) is also, to varying degrees, influenced by environmental factors.

The term genotype was first used by Danish geneticist Wilhelm Johannsen (1857–1927) to describe the entire genetic or hereditary constitution of an organism, In contrast, Johannsen described displayed characters or traits (e.g., anatomical traits, biochemical traits, physiological traits, etc.) as an organism's phenotype.

Genotype and phenotype represent very real differences between genetic composition and expressed form. The genotype is a group of genetic markers that describes the particular forms or variations of genes (alleles) carried by an individual. Accordingly, an individual's genotype includes all the alleles carried by that individual. An individual's genotype, because it includes all of the various alleles carried, determines the range of traits possible (e.g., a individual's potential to be afflicted with a particular disease). In contrast to the possibilities contained within the genotype, the phenotype reflects the manifest expression of those possibilities (potentialities). Phenotypic traits include obvious observable traits as height, weight, eye color, hair color, etc. The presence or absence of a disease, or symptoms related to a particular disease state, is also a phenotypic trait.

A clear example of the relationship between genotype and phenotype exists in cases where there are dominant and recessive alleles for a particular trait. Using an simplified monogenetic (one **gene**, one trait) example, a capital "T" might be used to represent a dominant allele at a particular locus coding for tallness in a particular plant, and the lowercase "t" used to represent the recessive allele coding for shorter plants. Using this notation, a diploid plant will possess one of three genotypes: TT, Tt, or tt (the variation tT is identical to Tt). Although there are three different genotypes, because of the laws governing dominance, the plants will be either tall or short (two phenotypes). Those plants with a TT or Tt genotype are observed to be tall (phenotypically tall). Only those plants that carry the tt genotype will be observed to be short (phenotypically short).

In humans, there is genotypic sex determination. The genotypic variation in sex **chromosomes**, XX or XY decisively determines whether an individual is female (XX) or male (XY) and this genotypic differentiation results in considerable phenotypic differentiation.

Although the relationships between genetic and environmental influences vary (i.e., the degree to which genes specify phenotype differs from trait to trait), in general, the more complex the biological process or trait, the greater the influence of environmental factors. The genotype almost completely directs certain biological processes. Genotype, for example, strongly determines when a particular tooth develops. How long an individual retains a particular tooth, is to a much greater extent, determined by environmental factors such diet, dental **hygiene**, etc.

Because it is easier to determine observable phenotypic traits that it is to make an accurate determination of the relevant genotype associated with those traits, scientists and physicians place increasing emphasis on relating (correlating) phenotype with certain genetic markers or genotypes.

There are, of course, variable ranges in the nature of the genotype-environment association. In many cases, genotype-environment interactions do not result in easily predictable phenotypes. In rare cases, the situation can be complicated by a process termed phenocopy where environmental factors produce a particular phenotype that resembles a set of traits coded for by a known genotype not actually carried by the individual. Genotypic frequencies reflect the percentage of various genotypes found within a given group (population) and phenotypic frequencies reflect the percentage of observed expres-

sion. Mathematical measures of phenotypic variance reflect the variability of expression of a trait within a population.

The exact relationship between genotype and disease is an area of intense interest to geneticists and physicians and many scientific and clinical studies focus on the relationship between the effects of a genetic changes (e.g., changes caused by **mutations**) and disease processes. These attempts at genotype/phenotype correlations often require extensive and refined use of statistical analysis.

See also Genetic code; Genetic identification of microorganisms; Genetic mapping; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Immunogenetics; Microbial genetics

GENTAMICIN • *see* ANTIBIOTICS

GERM THEORY OF DISEASE

The germ theory is a fundamental tenet of medicine that states that **microorganisms**, which are too small to be seen without the aid of a **microscope**, can invade the body and cause certain diseases.

Until the acceptance of the germ theory, diseases were often perceived as punishment for a person's evil behavior. When entire populations fell ill, the disease was often blamed on swamp vapors or foul odors from sewage. Even many educated individuals, such as the prominent seventeenth century English physician William Harvey, assumed that **epidemics** were caused by miasmas, poisonous vapors created by planetary movements affecting the Earth, or by disturbances within the Earth itself.

The development of the germ theory was made possible by certain laboratory tools and techniques that permitted the study of **bacteria** during the seventeenth and eighteenth centuries. The invention of primitive microscopes by the English scientist **Robert Hooke** and the Dutch merchant and amateur scientist **Anton van Leeuwenhoek** in the seventeenth century, gave scientists the means to observe microorganisms. During this period, a debate raged among biologists regarding the concept of spontaneous generation.

Until the second part of the nineteenth century, many scientists held that some lower life forms could arise spontaneously from nonliving matter, for example, flies from manure and maggots from decaying corpses. In 1668, however, the Italian physician Francisco Redi demonstrated that decaying meat in a container covered with a fine net did not produce maggots. Redi asserted this was proof that merely keeping egg-laying flies from the meat by covering it with a net while permitting the passage of air into the containers was enough to prevent the appearance of maggots. However, the belief in spontaneous generation remained widespread even in the scientific community.

In the 1700s, more evidence that microorganisms can cause certain diseases was passed over by physicians, who did not make the connection between **vaccination** and microorganisms. During the early part of the eighteenth century, Lady Montague **(Mary Wortley Montague)**, wife of the British ambassador to that country, noticed that the women of Constantinople routinely practiced a form of **smallpox** prevention that included "treating" healthy people with pus from individuals suffering from smallpox. Lady Montague noticed that the Turkish women removed pus from the lesions of smallpox victims and inserted a small amount of it into an area of broken skin of the recipients.

While the practice generally caused a mild form of the illness, many of these same people remained healthy while others succumbed to smallpox epidemics. The reasons for the success of this preventive treatment, called variolation, were not understood at the time, and depended on the coincidental use of a less virulent smallpox virus and the fact that the virus was introduced through the skin, rather than through its usually route of entry, the respiratory tract.

Lady Montague introduced the practice of variolation to England, where physician **Edward Jenner** later modified and improved the technique. Jenner noticed that milkmaids who contracted **cowpox** on their hands from touching the lesions on the udders of cows with the disease rarely got smallpox. He showed that inoculating people with cowpox prevented smallpox. The success of this technique, which demonstrated that the identical substances need not be used to stimulate the body's protective mechanisms, still did not convince many scientists of the existence of disease-causing microorganisms.

Thus, the debate continued well into the 1800s. In 1848, **Ignaz P. Semmelweis**, a Hungarian physician working in German hospitals, discovered that a sometimes-fatal infection commonly found in maternity hospitals in Europe could be prevented by simple **hygiene**. Semmelweis demonstrated that medical students doing autopsies on the bodies of women who died from puerperal fever often spread that disease to maternity patients they subsequently examined. He ordered these students to wash their hands in chlorinated lime water before examining pregnant women. Although the rate of puerperal fever in his hospital plummeted dramatically, many other physicians continued to criticize this practice as time-consuming and useless.

In 1854, modern **epidemiology** was born when the English physician John Snow determined that the source of cholera epidemic in London was the contaminated water of the Broad Street pump. After he ordered the pump closed, the epidemic ebbed. Nevertheless, many physicians refused to accept that invisible organisms could spread disease. The argument took an important turn in 1857, however, when the French chemist **Louis Pasteur** discovered "diseases" of wine and beer. French brewers asked Pasteur to determine why wine and beer sometimes spoiled. Pasteur showed that, while yeasts produce alcohol from the sugar in the brew, bacteria could change the alcohol to vinegar. His suggestion that brewers heat their product enough to kill bacteria but not **yeast**, was a boon to the brewing industry, a process called **pasteurization**. In addition, the connection Pasteur made between food spoilage and microorganisms was a key step in demonstrating the link between microorganisms and disease. Pasteur

observed that, "There are similarities between the diseases of animals or man and the diseases of beer and wine." The notion of spontaneous generation received another blow in 1858, when the German scientist Rudolf Virchow introduced the concept of biogenesis. This concept holds that living cells can arise only from preexisting living cells. This was followed in 1861 by Pasteur's demonstration that microorganisms present in the air can contaminate solutions that seemed sterile. For example, boiled nutrient media left uncovered will become contaminated with microorganisms, thus disproving the notion that air itself can create microbes.

In his classic experiments, Pasteur first filled short-necked flasks with beef broth and boiled them. He left some opened to the air to cool and sealed others. The sealed flasks remained free of microorganisms, while the open flasks were contaminated within a few days. Pasteur next placed broth in flasks that had open-ended, long necks. After bending the necks of the flasks into S-shaped curves that bent downward, then swept sharply upward, he boiled the contents. Even months after cooling, the uncapped flasks remained uncontaminated. Pasteur explained that the S-shaped curve allowed air to pass into the flask; however, the curved neck trapped airborne microorganisms before they could contaminate the broth.

Pasteur's work followed earlier demonstrations by both himself and Agostino Bassi, an amateur microscopist, showing that silkworm diseases can be caused by microorganisms. While these observations in the 1830s linked the activity of microorganisms to disease, it was not until 1876 that German physician **Robert Koch** proved that bacteria could cause diseases. Koch showed that the bacterium *Bacillus anthracis* was the cause of **anthrax** in cattle and sheep, and he discovered the organism that causes **tuberculosis**.

Koch's systematic methodology in proving the cause of anthrax was generalized into a specific set of guidelines for determining the cause of infectious diseases, now known as **Koch's postulates**. Thus, the following steps are generally used to obtain proof that a particular organism causes a particular disease:

1. The organism must be present in every case of the disease.
2. The organism must be isolated from a host with the corresponding disease and grown in pure **culture**.
3. Samples of the organism removed from the pure culture must cause the corresponding disease when inoculated into a healthy, susceptible laboratory animal.
4. The organism must be isolated from the inoculated animal and identified as being identical to the original organisms isolated from the initial, diseased host.

By showing how specific organisms can be identified as the cause of specific diseases, Koch helped to destroy the notion of spontaneous generation, and laid the foundation for modern medical microbiology. Koch's postulates introduced what has been called the "Golden Era" of medical bacteriology. Between 1879 and 1889, German microbiologists isolated the organisms that cause cholera, **typhoid fever**, **diphtheria**, **pneumonia**, **tetanus**, **meningitis**, **gonorrhea**, as well the *Staphylococcus* and *Streptococcus* organisms.

Louis Pasteur, one of the "fathers" of microbiology. Among his accomplishments was the demonstration of the involvement of microorganisms in disease.

Even as Koch's work was influencing the development of germ theory, the influence of the English physician **Joseph Lister** was being felt in operating rooms. Building on the work of both Semmelweis and Pasteur, Lister began soaking surgical dressings in carbolic acid (phenol) to prevent postoperative infection. Other surgeons adopted this practice, which was one of the earliest attempts to control infectious microorganisms.

Thus, following the invention of microscopes, early scientists struggled to show that microbes can cause disease in humans, and that **public health** measures, such as closing down sources of **contamination** and giving healthy people vaccines, can prevent the spread of disease. This led to reduction of disease transmission in hospitals and the community, and the development of techniques to identify the organisms that for many years were considered to exist only in the imaginations of those researchers and physicians struggling to establish the germ theory.

See also Bacteria and bacterial infection; Bacterial growth and division; Centers for Disease Control (CDC); Colony and colony formation; Contamination, bacterial and viral; Epidemics and pandemics; Epidemics, bacterial; Epidemics, viral; Epidemiology, tracking diseases with technology;

Scanning electron micrograph of *Giardia*.

History of microbiology; History of public health; History of the development of antibiotics; Infection and resistance; Laboratory techniques in microbiology

GIARDIA AND GIARDIASIS

Giardia is a protozoan parasite that can be transmitted to humans via drinking water that is contaminated with feces. The prototypical species is *Giardia lamblia*. The protozoan causes an intestinal malady, typified by diarrhea that is called giardiasis. The intestinal upset has also been dubbed "beaver fever."

The natural habitat of *Giardia* is the intestinal tract of warm-blooded animals. In the wild, warm-blooded creatures such as beavers and bears are natural reservoirs of the protozoan. Also, domestic dogs and cats can harbor the microbe. Typically, *Giardia* is passed onto humans by the fecal **contamination** of drinking water by these animals. The ingestion of only a few cysts is sufficient to establish an infection

Giardia has two distinct morphologies. In the environment, such as in water, *Giardia* is in the form of what is termed a cyst. An individual cyst is egg-shaped and contains four eye-like appearing nuclear bodies. This form is functionally analogous to a bacterial spore. It is a dormant form of the organism that is designed to allow preservation of the genetic material in a hostile environment. Cysts can remain capable of growth for months in water.

In the more hospitable intestinal tract, *Giardia* reverts to an actively growing and dividing form that is termed a trophozoite. A trophozoite has a distinctive "tear-drop" shape and flagella protruding from five regions on the surface. Two nuclei present the appearance of eyes and a darker central body looks somewhat like a mouth. The effect is to produce an image in the light **microscope** that is reminiscent of a face.

When excreted from an animal into water, the cyst form is particularly insidious because of the small size, which can allow the cyst to elude filtering steps in a drinking water treatment plant. Also, the cyst is resistant to chlorine, which is the most common means of disinfecting drinking water. Other documented routes of fecal to oral transmission are the sharing of toys in day care facilities (where hands are soiled) and via oral/anal sexual acts. Food borne transmission can occur, but is rare.

While in some people an infection with *Giardia* does not produce symptoms, many people experience prolonged diarrhea. Indeed, giardiasis is a major cause of intestinal upset

in the world. In North America, giardiasis is the leading cause of non-bacterial diarrhea. The diarrhea typically persists for a few weeks to a few months, although in extreme cases the infection can persist for years. Infection produces a general malaise and considerable weight loss. The diarrhea tends to be mushy but, in contrast to the diarrhea produced in bacterial and amoebic **dysentery**, it is not bloody. Other symptoms of giardiasis include flatulence, sore abdomen, foul-smelling breath and, particularly in infants, the disruption of normal body growth. Research on animal models has also demonstrated that the infection disrupts the ability of the intestinal epithelial cells to absorb nutrients from the intestinal contents. The decreased absorption of compounds such as vitamin B_{12} and lactose can have deleterious effects on overall health.

The molecular basis of the infection is still not fully resolved. However, the trophozoite form of the protozoan is required, as is associated with the surface of the intestinal epithelial cells. In contrast to another intestinal disease causing microbe, *Entamoeba histolytica*, *Giardia lamblia* does not invade the host tissue. Studies with animal models have indicated that the symptoms of giardiasis may be due to a physical barrier to the absorption of nutrients, the disruption of intestinal structures called microvilli by the adherent **protozoa**, and production of a toxin that damages the epithelial cells.

During an active infection, trophozoites undergo division to new daughter trophozoites. Also, formation of cysts occurs and the cysts are excreted with the feces in huge numbers. These can be passed onto someone else to establish a new infection.

Treatment for giardiasis can include the use of **antibiotics**. Often, however, the malady is self-limiting without intervention. Prevention of giardiasis is a more realistic option, and involves proper treatment of drinking water and good hygienic practices, especially handwashing.

Currently, the detection of *Giardia* is based on the microscopic detection of either form of the protozoan, although animal models of the infection are being researched. The infection has been produced in gerbils. The lack of a routine detection method is problematic for water treatment. The need for rapid testing of drinking water for *Giardia* is pressing as the incidence of infection is increasing, with the encroachment of human habitation on previously pristine areas.

See also Amebic dysentery; Parasites; Water quality

GLIDING BACTERIA

Gliding **bacteria** is a term that refers to any bacteria that exhibit a gliding or creeping type of movement (also known as motility) when in contact with a solid surface.

There are hundred of types of gliding bacteria. Most are beneficial or benign to humans and animals. Some strains, such as the myxobacteria, produce **antibiotics** and compounds that act on tumors. In addition, some strains of gliding bacteria can degrade compounds, and so have potential in the biodegradation of pollutants. However, some strains are a major health concern. For example, gliding bacteria that live in human saliva can cause gum disease, and can be life-threatening if they enter the bloodstream. Other types of gliding bacteria cause disease in animals and fish.

The gliding motility of myxobacteria is only one of several forms the organism can adopt. Another form consists of a stalk with fruiting bodies positioned at one end. When exhibiting gliding motility, a single cell can be in motion or a population of cells can move in concert with one another. The latter type of gliding seems to require the group of cells. If a cell moves out in front of the others, the lead cell will soon stop. The nature of the communal movement is unknown.

The gliding motility of bacteria such as those in the myxobacteria, the green nonsulfur group of bacteria (such as *Chloroflexus auranticus*) and the nonphotosynthetic gliding bacteria (such as *Herpetosiphon*) has long been a fascination to bacteriologists. The bacteria glide smoothly with no evidence of cellular involvement in the movement. In fact, the gliding motion may be the result of what have been termed "slime fibrils," a complex of proteins, which are attached to the bacterium at one end and to the solid surface at the other end. The exact mechanism of action of the slime fibrils is still unresolved. If the fibrils have to move to propel a bacterium along, the nature of this movement and how the movement is powered remain unknown.

Some gliding bacteria are known to exhibit a chemotactic response, that is, a concerted movement either in the direction of an attractant or concerted movement away from a repellent. The chemical sensing system must be coordinated with the gliding mechanism. Again, the nature of this coordination is unresolved.

See also Bacterial movement

GLOBULINS

Globulins are immune molecules that are produced by the **immune system** in response to invasion of the body of agents that are perceived by the system as being foreign. Some immune globulins are also known as antibodies. Examples of **microorganisms** that stimulate globulin production are **bacteria**, **viruses**, molds, and **parasites**.

The production of globulins is triggered by the presence of the foreign material (antigen). Through an intricately coordinated series of events, the immune system responds to the presence of an antigen by the production of a corresponding globulin. There are three divisions of globulins, based on the movement of the molecules through a gel under the influence of an electric current (in other words, based on their size and their charge character). The three types are alpha, beta, and gamma globulins.

Alpha globulins are manufactured mainly in the liver. There are a number of so-called alpha-1 and alpha-2 globulins. The functions of these globulins includes the inhibition of an enzyme that digests protein, an inhibitor of two compounds vital in the clumping (coagulation) of blood, and a protein that can transport the element copper.

Alpha and beta globulins function as **enzymes** and proteins that transport compounds in the body. Gamma globulins act as the **antibody** defense against antigen invasion.

Beta globulins are also manufactured predominantly in the liver. Akin to the alpha type globulins, there are beta-1 and beta-2 globulins. Examples of beta globulins include a protein that binds iron in the body, factors involved with the immune targeting of foreign material for immune destruction, and a species of immunoglobulin antibody termed IgM.

Gamma globulins are manufactured in cells of the immune system known as lymphocytes and plasma cells. These globulins, which are known as IgM, IgA, and IgG, represent antibodies. Depending on the nature of the invading antigen, a specific immun globulin will be produced in great numbers by a specific lymphocyte or plasma cell. Infection with a different antigen will stimulate the production of a different immune globulin. As the infection eases, the production of the immun globulin will decrease.

The quantities of the various globulins in the blood can be diagnostic of malfunctions in the body or specific diseases. Examples of maladies that affect the globulin levels are chronic microbial infections, liver disease, **autoimmune disorders**, leukemias, and rheumatoid arthritis.

See also Immunity: active, passive, and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunochemistry; Immunogenetics; Immunoglobulins and immunoglobulin deficiency syndromes; Immunologic therapies; Immunological analysis techniques; Laboratory techniques in immunology

GLYCOCALYX

The glycocalyx is a carbohydrate-enriched coating that covers the outside of many eukaryotic cells and prokaryotic cells, particularly **bacteria**. When on eukaryotic cells the glycocalyx can be a factor used for the recognition of the cell. On bacterial cells, the glycocalyx provides a protective coat from host factors. The possession of a glycocalyx on bacteria is associated with the ability of the bacteria to establish an infection.

The glycocalyx of bacteria can assume several forms. If in a condensed form that is relatively tightly associated with the underlying cell wall, the glycocalyx is referred to as a capsule. A more loosely attached glycocalyx that can be removed from the cell more easily is referred to as a slime layer.

The bacterial glycocalyx can vary in structure from bacteria to bacteria. Even particular bacteria can be capable of producing a glycocalyx of varying structure, depending upon the growth conditions and nutrients available. Generally, the glycocalyx is constructed of one or more sugars that are called saccharides. If more than one saccharide is present, the glycocalyx is described as being made of polysaccharide. In some glycocalyces, protein can also be present.

There are two prominent functions of the glycocalyx. The first function is to enable bacteria to become harder for the immune cells called phagocytes so surround and engulf. This is because the presence of a glycocalyx increases the effective diameter of a bacterium and also covers up components of the bacterium that the **immune system** would detect and be stimulated by. Thus, in a sense, a bacterium with a glycocalyx becomes more invisible to the immune system of a host.

Infectious strains of bacteria such as *Staphylococcus, Streptococcus,* and *Pseudomonas* tend to elaborate more glycocalyx than their corresponding non-infectious counterparts.

The second function of a bacterial glycocalyx is to promote the adhesion of the bacteria to living and inert surfaces and the subsequent formation of adherent, glycocalyx-enclosed populations that are called **biofilms**. Biofilm bacteria can become very hard to kill, party due to the presence of the glycocalyx material. Many persistent infections in the body are caused by bacterial biofilms. One example is the dental **plaque** formed by glycocalyx-producing *Streptococcus mutans*, which can become a focus for tooth enamel-digesting acid formed by the bacteria. Another example is the chronic lung infections formed in those afflicted with certain forms of cystic fibrosis by glycocalyx-producing *Pseudomonas aeruginosa*. The latter infections can cause sufficient lung damage to prove lethal.

See also Anti-adhesion methods; Bacterial surface layers

GOLGI, CAMILLO (1843-1926)

Italian histologist

Among other achievements in neurobiology, Camillo Golgi devised a method of staining nerve tissue using silver nitrate. Golgi-stained nerve tissue revealed unique structures with fine projections, which were later recognized as individual cells, or neurons.

Golgi was born in Corteno, Italy, on July 7, 1843. His hometown was later re-named Corteno-Golgi in his honor. Golgi studied medicine at the University of Pavia, where he received his M.D. in 1865. After graduation, he worked briefly in a psychiatric clinic, but eventually decided to pursue a career in histological research.

Financial difficulties forced him in 1872 to accept a position as chief medical officer at the Hospital for the Chronically Ill in Abbiategrasso, Italy. No research facilities were available there, however, and he was able to continue his studies only by converting an unused kitchen into a laboratory. By 1875, Golgi had earned sufficient fame to receive an appointment as lecturer in histology at the University of Pavia. Four years later, he was appointed Professor of Anatomy at the University of Siena, but he stayed only a year there before returning to Pavia as Professor of Histology.

Golgi's earliest research involved the study of neurons, or nerve cells. Neurons present a number of problems for researchers that other cells do not. While most cells are compact and have a relatively fixed shape, neurons are commonly very long and thin with structures that are difficult to see clearly. In the 1860s, techniques used to stain and study non-nerve cells were well developed, but they were largely useless with neurons. As a result, a great deal of uncertainty surrounded the structure and function of neurons and neuron networks.

In 1873, Golgi found that silver salts could be used to dye neurons. The neurons turned black and stood out clearly from surrounding tissue. Golgi perfected his technique so that the addition of just the right amount of dye for just the right period of time would highlight one or another part of the neuron, a single complete neuron, or a group of neurons.

Golgi's new technique resolved some questions about the nervous system, but not all. He was able, for example, to confirm the view of Wilhelm von Waldeyer-Hartz that neurons are separated by narrow gaps, synapses, and are not physically connected to each other. He was not able to completely explain, however, the complex, overlapping network of dendrites.

While studying the brain of a barn owl in 1896, Golgi made a second important discovery. He found previously undetected bodies near the nuclear membrane. The function of those bodies, now known as Golgi apparatus, or **Golgi bodies**, is still not understood. For his research on the nervous system, Golgi was awarded a share of the 1906 Nobel Prize for physiology or medicine.

Between 1885 and 1893, Golgi was also involved in research on **malaria**. He made one especially interesting discovery in this field, namely that all the malarial **parasites** in an organism reproduce at the same time, a time that corresponds to the recurrence of fever.

In addition to his scientific work, Golgi was active in Italian politics. He was elected a Senator in 1900 and served in a number of administrative posts at Pavia. Golgi died in Pavia on January 21, 1926.

See also Cell cycle and cell division; Cell membrane transport; Golgi body; Malaria and the physiology of parasitic inflections

GOLGI BODY

The Golgi body, or Golgi apparatus is a collection of flattened membrane sacks called cisternae that carry out the processing, packaging, and sorting of a variety of cellular products in higher plants and animals. This important cellular organelle was named in honor of **Camillo Golgi**, the Italian neuroanatomist who first described it in brain cells late in the nineteenth century. An individual Golgi apparatus is usually composed of four to eight cisternae, each a micron or less in diameter stacked on top of each other like pancakes. Many cisternal stacks interconnected by tubules and mobile transport vesicles make up a Golgi complex, which often is located near the **nucleus** in the center of the cell. In some animal cells, this complex can be huge, filling much of the cytoplasmic space. In some plant cells, on the other hand, many small, apparently independent Golgi apparatuses are distributed throughout the cell interior.

Each Golgi stack has a distinct orientation. The *cis* or entry face is the site at which transport vesicles bringing newly synthesized products from the endoplasmic reticulum dock with and add their contents to the Golgi cisternae. A complex network of anastomosing (connecting) membrane tubules attach to and cover the fenestrated cisternae on the *cis* face and serves as a docking site for transport vesicles. From the *cis* face a flow of vesicles carry transport and chaperone proteins back to the endoplasmic reticulum, while secretory products move on into the medial cisternae where further processing takes place. Finally, the products move to the *trans* or exit face where they undergo final processing, sorting, and packaging into vesicles that will carry them to the cell surface for secretion or to other cellular organelles for storage or use. Complex oligosaccharides are synthesized in the Golgi apparatus, and glycoproteins are assembled as materials move through the compartments of this organelle. A unique set of **enzymes** and chaperone proteins occur in each of the Golgi compartments to direct and carry out this complex set of reactions.

See also Cell cycle and cell division; Cell membrane transport

GONORRHEA

Gonorrhea is among the most common **sexually transmitted diseases** (STD) and is also among the most common bacterial infections in adults. In the United States, between 2.5 and 3 million cases are reported each year, most occurring in people under age 30. In its early stages, gonorrhea may cause no symptoms and therefore, can be spread by unsuspecting victims. In females, gonorrhea often remains asymptomatic but can lead to vaginal itching, discharge, or uterine bleeding and other serious complications. An infected woman who gives birth can transmit the disease to her infant, often resulting in childhood blindness. As a precaution, silver nitrate is routinely administered to the eyes of newborns to prevent this condition. In males, gonorrhea causes infection of the urethra and painful urination. Though not deadly, the disease if untreated can infect other genital organs. If the infection spreads throughout the blood stream, it can cause an arthritis-dermatitis syndrome.

Gonorrhea was described in early writings from Egypt, China, and Japan. Warnings against "unclean discharge from the body" appear in the Bible. A diagnostic description of the disease was written in the Middle Ages. In the late fifteenth century, a **syphilis** epidemic raged throughout Europe, though at that time, syphilis was often confused with gonorrhea and some physicians assumed that gonorrhea was the first stage of syphilis. The gram-negative bacterium that causes gonorrhea was discovered in 1879 by Albert Neisser (1855–1916), a German physician who went on to identify the bacterial cause for **leprosy**. German immunologist **Paul Ehrlich** named the bacterium *Gonococcus*. Since then, five types of the *Gonococcus* organism have been identified.

A test for the presence of *Gonococcus* bacterium serves as the diagnostic tool. The first effective treatments for gonorrhea were the sulfonamides, which became available in 1937. During World War II, **penicillin** became widely available for the treatment of gonorrhea and other bacterial disease. However, while penicillin and related **antibiotics** are effective in about 90% of cases, some strains of the *Gonococcus* are becoming resistant to penicillin.

See also History of the development of antibiotics; History of public health

GOODPASTURE, ERNEST WILLIAM (1886-1960)

American pathologist

Ernest William Goodpasture created a means of culturing **viruses** in the laboratory without **contamination** from foreign **bacteria**. This research was instrumental in the development of most of the vaccines and inoculations used in medicine today. Additionally, Goodpasture developed an alternate way of staining specimens for examination under the **microscope**. He also pursued research interests in varying other fields of medicine, and identified a progressive and rare **immune system** illness that became known as Goodpasture's Syndrome.

Goodpasture was born in Montgomery County, Tennessee. He left home to study medicine in 1909, and received his doctorate from Johns Hopkins University. He then served as the Rockefeller Fellow in Pathology until 1914. Goodpasture returned to practicing medicine and served his residency at Peter Bent Brigham Hospital in Boston from 1915 to 1917. In 1917, he was offered a professorship at Harvard University, but only remained there for three years. Shortly thereafter, Goodpasture was appointed chair of pathology at Vanderbilt University. The position at Vanderbilt afforded Goodpasture the opportunity to return home, he accepted and remained with the university for the entirety of his career.

In 1931, Goodpasture, working with Alice Woodruff, devised a method for cultivating viruses that revolutionized **virology**. Because rickettsiae and other viruses will only grow on living tissue, scientists had to study viruses on living hosts or on tissue cells cultures in the lab. Before the advent of **antibiotics**, lab cultures were often tainted by bacteria. Thus, viruses remained illusive to scientific study. Little was known about their structure and behavior. Goodpasture used fertilized chicken eggs to **culture** his viruses, a method that proved not only successful, but also cost effective. The team first successfully cultured fowl pox virus, but quickly proved that a multitude of viruses could be studied using the technique. Within a span of a few years, other scientists used Goodpasture's technique to create vaccines for **yellow fever**, **smallpox**, and **influenza**.

Goodpasture himself worked to create a **vaccination** against the **mumps**. In 1934, Goodpasture and his colleague, C.D. Johnsen, proved that the mumps virus vas filterable. This prompted to team to devise a method by which the virus could be manipulated to produce a **vaccine**.

Throughout the course of his career, Goodpasture was chiefly concerned with infectious diseases, but he also conducted research in the formation of various types of cancers and genetic diseases. His name was given to a condition that he discovered working in tandem with doctors at Vanderbilt University Hospital. Goodpasture's Syndrome is a rare and often fatal autoimmune disorder that affects the kidneys.

See also Laboratory techniques in immunology; Laboratory techniques in microbiology

GOTSCHLICH, EMIL CLAUS (1935-)

German-American physician and bacteriologist

Emil Gotschlich's basic research on *Meningococcus, Gonococcus, Streptococcus, Haemophilus, Escherichia coli,* protein antigens, and polysaccharides has contributed much to the knowledge of **immunology**, vaccines, and the bacterial pathogenesis of **meningitis**, **gonorrhea**, and other diseases.

Gotschlich was born on January 17, 1935, in Bangkok, Thailand, to Emil Clemens Gotschlich, and his wife Magdalene, née Holst, both expatriate Germans. He immigrated to the United States in 1950 and became a naturalized American citizen in 1955, the same year that he received his A.B. from the New York University College of Arts and Sciences. After receiving his M.D. from the New York University School of Medicine in 1959, he interned at Bellevue Hospital in New York City until 1960, then joined the staff of Rockefeller University, where he built the rest of his career, except for serving as a captain in the Department of Bacteriology of the U.S. Army Medical Corps at the Walter Reed Institute for Research in Washington, D.C., from 1966 to 1968. At Rockefeller in 2002, he is simultaneously professor of bacteriology, head of the Bacterial Pathogenesis and Immunology Laboratory, vice president for medical sciences, and principal investigator of the General Clinical Research Center.

Gotschlich's team at Rockefeller continues to achieve important results in bacteriology and immunology and has published hundreds of scientific papers. As of 2000, Gotschlich was the author, lead author, or co-author of 135 of these papers. The main focus of his research is the *Neisseria* genus of **bacteria**, especially two pathogenic varieties: the meningococcus *Neisseria meningitidis* and the gonococci *Neisseria gonorrhoeae*. In the 1970s, he engaged in a fruitful scientific correspondence with **Harry A. Feldman** (1914–1985) about meningococcal diseases and won the 1978 Albert Lasker Award for his part in developing a polysaccharide **vaccine** against these diseases. In 1984, he outlined protocols for the development of a gonorrhea vaccine. In 2001, the United States **Centers for Disease Control** appointed him to its national review committee on **anthrax** vaccine safety and efficacy.

Medicine and medical research are a tradition in Gotschlich's family. His grandfather, Emil Carl Anton Constantin Gotschlich, a student of Carl Flügge and a colleague of Max Josef von Pettenkoffer (1818–1901) and **Robert Koch** (1843–1910), was a prominent German academic physician, hygienist, and epidemiologist who specialized in cholera and tropical diseases. His great uncle, Felix Gotschlich (b. 1874), also studied cholera and in 1906 isolated *El Tor vibrio cholerae,* an epidemic strain of the cholera bacillus. His father was a physician in private practice in Thailand. His second wife, Kathleen Ann Haines (b. 1949), a pediatric allergist and immunologist in New York City; his son, Emil Christopher Gotschlich (b. 1961), an obstetrician/gynecologist in Portland, Maine; and his daughter, Hilda Christina Gartley (b. 1965), a pediatrician in Boston, Massachusetts, all continue this tradition.

See also Antibiotics; Antibody and antigen; Bacteria and

bacterial infection; Haemophilus; Infection control; Lipopolysaccharide and its constituents; Meningitis, bacterial and viral; Microbiology, clinical; Serology; Streptococci and streptococcal infections; Vaccine

Gram, Hans Christian Joachim (1853-1938)

Danish pharmacologist

Hans Christian Joachim Gram was a Danish physician and bacteriologist who developed the most widely used method of staining bacterial cells for microscopic study.

Gram was born in Copenhagen, Denmark, on September 13, 1853. He received a B.A. in the natural sciences from the Copenhagen Metropolitan School in 1871 and served as an assistant to the zoologist Japetus Steenstrup from 1873 to 1874. He subsequently became interested in medicine and earned a medical degree from the University of Copenhagen in 1878. Gram, who worked in several areas of science and medicine, earned a gold medal in 1882 for a study on human erythrocytes. The following year he received a doctoral degree for his work in this field.

After obtaining his degree, Gram pursued post-doctoral studies in Berlin, focusing on bacteriology and pharmacology. It was in Berlin in 1884 that he published his work on the technique of staining cells, a procedure that became widely known as **Gram staining**.

At that time, the method of staining cells was not entirely new to scientific research and several methods were already being used. Gram borrowed from a procedure initially devised by **Paul Ehrlich**, who used alkaline aniline solutions to stain **bacteria** cells. Experimenting with pneumococci bacteria, Gram first applied Gentian violet, which stained the cells purple, and then washed the cells with Lugol's solution (iodine), which served as a mordant to fix the dye. He followed those steps by applying alcohol, which washed away any dye that was not permanently fixed. Gram found that some cells remained purple (Gram positive), while others stayed essentially unstained (Gram negative). Gram's method aided microscopic study of bacteria, as well as provided a means of differentiating and classifying bacteria cells. Several years later, the pathologist Carl Weigart improved upon Gram's method by adding another staining step, which consisted in dyeing the Gram-negative cells with saffranine.

Gram remained in Berlin working as an assistant in a hospital until 1891, when he was appointed as a professor of pharmacology at the University of Copenhagen. In 1889, Gram married Louise I. C. Lohse, and in 1892, advanced to the position of chief of internal medicine at the Royal Frederiks Hospital. Extremely active in the field of medical education, Gram also maintained a large internal medicine practice. From 1901 to 1921, Gram served as chairman of the Pharmacopoeia Commission, during which time he abolished the use of many useless and obsolete therapeutic treatments. In addition, he published a four-volume book on the importance of rational pharmacology in clinical science. After his retirement in 1923, he returned to an earlier interest: the history of medicine. During his career, Gram received several honors including the Danneborg Commander's Cross, the Golden Medal of Merit, and an honorary M.D. Gram died in Copenhagen, on November 14, 1938.

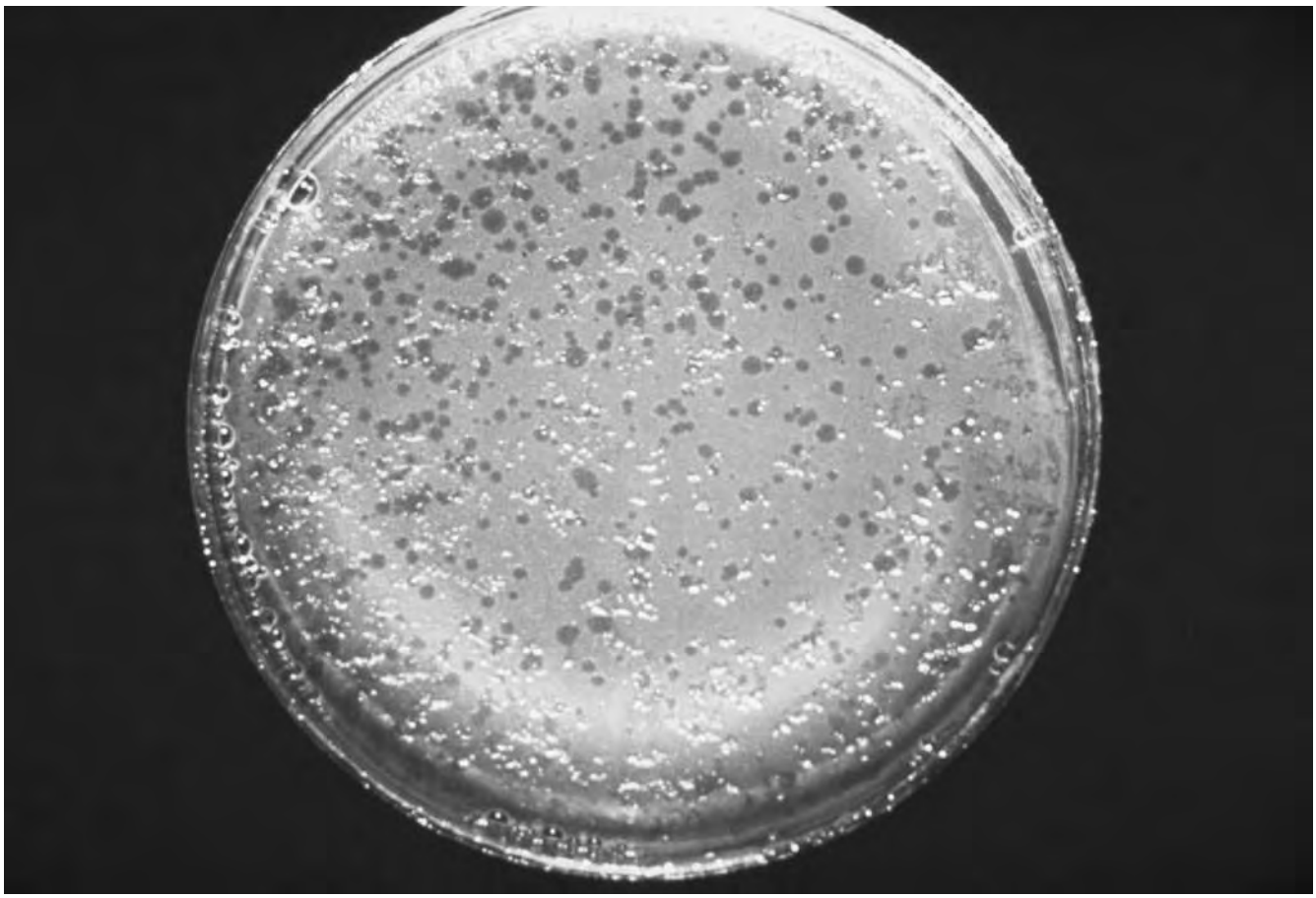
See also Laboratory techniques in microbiology

Gram staining

In the second half of nineteenth century, scientists proved that specific bacterial organisms caused specific diseases, and the field of microbiology was on its way to becoming a distinct science. The **microscope** was also further developed during that time, and scientists were concerned with identifying and classifying **bacteria**. Most bacteria are difficult to see with a compound microscope, but can be seen when there is obvious contrast between the bacteria cells and their surrounding medium. Various dyes are used to stain cells so that they are more easily seen. As early as the late eighteenth century, scientists had developed some basic methods of staining cells to aid in their study and used natural substances such as saffron, which stained some parts of a cell. The discovery of synthetic dyes in the mid-1800s enabled scientists to utilize more colors to stain cells.

In 1884, the Danish physician **Hans Christian Joachim Gram** further developed a method of staining bacteria originally developed by the German biologist **Paul Ehrlich**. Ehrlich used aniline water and gentian violet (a cationic dye) to stain cells, and the cell walls would appear purple after staining. Gram added a potassium triiodide solution, which acted as a mordant for the gentian violet dye, and then poured ethanol over the cells to wash away the unfixed dye. Gram found that some of the cells remained purple, while others did not. Bacteria that remained purple were termed positive and those that did not remain purple were called negative. A few years later, Carl Weigert, director of the Senckberg Foundation in Frankfurt, Germany, added another step to the staining method. Weigert followed Gram's procedure with a final staining using saffranine (an anionic dye), which subsequently stained the negative bacteria red. The Gram stain is still considered the definitive, differential test to determine the chemical make-up of a bacterium cell wall. On the basis of a cell's reaction to the Gram stain, bacteria are divided into two groups, Gram positive and Gram negative.

The distinguishing feature between Gram positive and negative bacteria is the difference in the structure of the cell walls. The cell wall of a Gram positive is a thick, single layer of a cross-linked polysaccharide that is easily stained by gentian violet, while the cell wall of a Gram negative bacterium consists of a thin layer of polysaccharide and is covered by a lipid layer that resists the gentian violet, but can be stained by saffranine. Many dyes, which are organic compounds, are positively charged and easily combine with the negatively charged, acidic polysaccharide wall. Other dyes are negatively charged and combine with protein-based cell constituents.



Growth of virus creates clearing in lawn of growing *Escherichia coli*.

The chemical make-up of the cell wall also determines the penetrability of the wall by various drugs. Knowing if a bacterium is Gram positive or negative determines what type of antibiotic is suitable for treatment, as some **antibiotics** act against Gram positive bacteria (i.e., **penicillin**), while others act against Gram negative bacteria (i.e., tetracycline or streptomycin). Another important consideration is the fact that some Gram negative bacteria release endotoxins, which can be fatal. When pharmaceutical companies develop new antibacterial drugs, the Gram stain is the method by which scientists determine the effectiveness of the drug.

See also Laboratory techniques in microbiology

GRAMICIDIN • *see* ANTIBIOTICS

GREEN FLUORESCENT PROTEIN • *see* LABORATORY TECHNIQUES IN IMMUNOLOGY

GROWTH AND GROWTH MEDIA

The ability of all organisms, including **microorganisms**, to grow and divide is the fundamental underpinning of their continued existence. In the laboratory, the nutrients needed for growth are supplied in the form of growth media.

Microorganisms such as **bacteria**, **yeast**, and algae grow by increasing in size, replicating their genetic material and other internal factors such as proteins, manufacturing the required additional cell wall material to enclose the new cell, and finally dividing to form two so-called daughter cells. Vital materials are required by most microbes, including carbon, hydrogen, nitrogen, phosphorus, calcium, cobalt, magnesium, and manganese. Often these elements must be supplied in the growth media, because the microbes cannot manufacture them. Once supplied, however, such elements form the building blocks upon which the microorganism can construct some (but not all) amino aids, proteins, and even the **DNA** (**deoxyribonucleic acid**) and **ribonucleic acid** (**RNA**) genetic materials.

Provision of the necessary elements is not sufficient to permit growth, however. The level of oxygen, moisture content, and temperature are examples of other factors that must be adjusted to permit the growth of the target microbe. For example, ***Escherichia coli*** requires a temperature of around

37° C [98.6° F], to match the temperature of its normal intestinal habitat. Refrigeration temperature, which does support the growth of some bacteria, does not support the growth of *Escherichia coli*.

The nature of the growth requirements of bacteria has been used as a means of grouping bacteria together. Photoautotrophic bacteria are those that use sunlight as a source of energy. **Heterotrophic bacteria** utilize organic carbon as a type of fuel for growth, while lithotrophic bacteria use inorganic carbon sources. As a final example, **autotrophic bacteria** are those bacteria that use carbon dioxide as the only source of carbon. Similarly, bacteria can be grouped according to their growth requirements with respect to temperature, salinity, the hydrogen ion concentration (also known as the **pH**), and oxygen.

In the laboratory, growth media often requires the presence of a few growth factors that the bacteria or other microbe cannot make themselves. For example, depending on the particular bacteria being grown, certain amino acids may need to be supplied. Likewise, the inclusion of compounds known as purines and pyrimidines may be necessary for the manufacture of DNA or RNA. Lastly, some vitamins may need to be added, which allow some **enzymes** to function. Sometimes bacteria can become altered so that the daughter cell has specific nutritional requirements that the parent cell does not. The mutant strain is referred to as an auxotroph. For example, a strain of *Escherichia coli* that requires the amino acid tryptophane for growth is a tryptophan auxotroph and is designated as *Escherichia coli trp*. Auxotrophs can be very useful as markers, or indicators of the success of an experimental procedure.

Growth of microorganisms can occur in a liquid growth medium, which is termed a broth, or on a solid medium. **Agar and agarose** are two examples of solid growth media. Often a broth can be supplemented with the solidifying agent to form **agar**. Growth media can be very nonspecific with respect to nutrients. For example, Brain-Hear Infusion (BHI) broth or agar is a blended mixture of animal brain and heart. A medium such as BHI is also referred to as a complex medium. Other media contains defined amounts of specific components. This type of medium is also called a minimal medium.

Growth media can also be tailored to favor the growth of one or a few types of bacteria over the many other types of bacteria that would develop on a nonselective medium, or to provide an enriched environment for those bacteria that would other wise grow poorly or very slowly. An example of a medium that is both selective and enriched is that used to grow bacteria in the genus *Halococcus*. These bacteria require very high concentrations of salt. The high sodium chloride concentration of the medium is lethal for all other types of bacteria.

In conventional broth and agar cultures, the growth of microorganisms is uncontrolled. The cells grow as fast as possible for as long as possible. However, growth of bacteria is possible such that the rate of growth and division can be controlled. Devices that accomplish this are the chemostat and the turbidostat. Comparison of the chemical make-up of the same type of microbe under relatively fast growing or slow growing conditions can be very useful, particularly because in infections, **bacterial growth** can be slower than in the laboratory environment.

See also Agar and agarose; Blood agar, hemolysis, and hemolytic reactions; Colony and colony formation; Synchronous growth

VON GRUBER, MAX (1853-1927)

Austrian physician and bacteriologist

Max von Gruber's discovery of specific bacterial agglutination in 1896 laid the groundwork for significant advances in **serology** and **immunology**.

Gruber was born in Vienna, the son of a prominent physician, Ignaz Gruber (1803–1872), and his wife, née Gabrielle Edle von Menninger. His brother, Franz von Gruber (1837–1918), became famous as an architect, military engineer, and teacher. After preparing for college at the Schottengymnasium in Vienna, Gruber studied chemistry and physiology at the University of Vienna, earned his M.D. there in 1876, then took postgraduate instruction in the biosciences under Max Josef von Pettenkoffer (1818–1901) in Vienna, Carl von Voit (1831–1908) and Carl Wilhelm von Nägeli (1817–1891) in Munich, Germany, and Carl Friedrich Wilhelm Ludwig (1816–1895) in Leipzig, Germany. Among his fellow graduate students under Pettenkoffer was Hans Buchner (1850–1902), who urged Gruber toward bacteriology.

Gruber began lecturing on **hygiene** at the University of Vienna in 1882, became professor of hygiene at the University of Graz, Austria, in 1884, and assumed the same position in 1887 at the University of Vienna, where he remained until 1902. He was promoted in 1891 to full professor. He was unhappy in Vienna because he considered the facilities ill kept and substandard. Nevertheless, he was able to attract to Vienna such stellar graduate students as future Nobel laureate **Karl Landsteiner** (1868–1943), Alois Lode (b. 1866), and Herbert Edward Durham (1866–1945). From 1902 until he retired in 1923, Gruber was director of the Institute for Hygiene, Munich.

In March, 1896, Gruber and Durham published a landmark article in a prestigious journal, *Münchener medizinische Wochenschrift* [Munich Medical Weekly], which described how **bacteria** of similar size clump together in sera, in ways specific to or determined by each serum. Their research concerned the typhoid bacillus *Salmonella typhi*, the cholera bacillus *Vibrio cholerae*, and the respective sera of typhoid and cholera patients. This clumping process, agglutination, soon had wider implications for serology, immunology, bacteriology, and clinical medicine. The first important practical consequence of Gruber's work on bacterial agglutination occurred in June 1896, when the French physician Georges Fernand Isidor Widal (1862–1929) developed a diagnostic agglutination test for typhoid, thereafter known as the Gruber-Widal test or the Gruber-Widal reaction.

In the first decade of the twentieth century, Gruber's main interest shifted toward right-wing social theory, political eugen-

ics, and so-called "racial hygiene." His *Hygiene des Geschlechtslebens* [Sexual Hygiene] first appeared in 1903, was reprinted or revised fifty-two times by 1925, and was translated into many languages, including English. With psychiatrist Ernst Rüdin (1874–1952), later a Nazi, Gruber co-edited *Fortpflanzung, Vererbung, und Rassenhygiene* [Propagation, Inheritance, and Racial Hygiene] in 1911. Thereafter, much of his work was political propaganda. Gruber attended Adolph Hitler's first big rally in 1921 and was impressed by Hitler's control of the crowd and command of issues. Gruber died in Berchtesgaden, Germany, on September 16, 1927.

See also Antibody-antigen, biochemical and molecular reactions; Bacteria and bacterial infection; History of immunology; Typhoid fever

H

HAEMOPHILUS

Haemophilus is a bacterial genus. The **bacteria** in this genus all share the characteristic of preferring to grow on solid laboratory media that contains blood cells. The blood supplies two factors that *Haemophilus* species require for growth. These are X factor and V factor. The utilization of these factors and of the blood cells causes the destruction of the cells and various characteristic reactions in the **blood agar**. Indeed, the name of the genus arise from these reactions.

Haemophilus are Gram negative in their **Gram staining** behavior and are very tiny rods in shape. The bacteria can display different shapes, and so is one of the types of bacteria known as pleomorphic bacteria. A hallmark of *Haemophilus* species is the formation of small colonies that are described as "satellites" around colonies of *Staphylococcus*.

In humans, *Haemophilus* is a normal resident of the throat and nose. However, spread of the bacteria beyond these sites can cause infections.

Haemophilus influenzae commonly infects children, causing a respiratory infection. This infection typically strikes those who already have the flu. The bacteria that cause these relatively severe reactions possess a **glycocalyx** that surrounds the each bacterium. The glycocalyx help thwart the host's immune response. Types of *Haemophilus influenzae* that cause less severe infections of the ears and the sinuses typically do not possess the glycocalyx.

Haemophilus influenzae infections can spread beyond the lungs. Spread to the central nervous system can result in an infection and **inflammation** of the sheath that surrounds nerve cells (**meningitis**). *Haemophilus influenzae* type b (which is also known as Hib) is particularly noteworthy in regard to meningitis. Hib can cause of fatal brain infection in young children.

Hib infections were once more common and dangerous. Now, however, the availability of a **vaccine** and the widespread requirement for a series of vaccinations early in life has greatly reduced the incidence of Hib meningitis.

Haemophilus can also spread to the airway. In that location, an infection known as epiglottits can be produced. The resulting obstruction of the airway in children less than 5 years of age can be fatal.

Other species of note include *Haemophilus aegyptius*, the cause of conjunctivitis (or pinkeye), a very contagious disease in children, and *Haemophilus ducreyi*, a sexually transmitted disease that causes genital ulceration.

See also Bacteria and bacterial infection; Blood agar, hemolysis, and hemolytic reactions

HALOPHILIC BACTERIA • *see* EXTREMOPHILES

HANCOCK, ROBERT ERNEST WILLIAM (1949-)

English bacteriologist

Robert (Bob) Hancock is a bacteriologist and professor of microbiology in the department of microbiology and **immunology** at the University of British Columbia (U.B.C.) in Vancouver, British Columbia, Canada. He is internationally renowned for his fundamental contributions to the study of **antibiotic resistance** of Gram-negative **bacteria**, particularly the antibiotic resistance of *Pseudomonas aeruginosa*.

Hancock was born in Merton, Surrey, England. Following his undergraduate education he obtained his Ph.D. at the University of Adelaide in Australia in 1974. Postdoctoral training followed at the University of Tübingen (1975–1977) and at the University of California at Berkeley (1977–1978). In 1978, Hancock became an assistant professor in the department of microbiology and immunology at U.B.C. In 1983, he became an associate professor with tenure, and became an associate member of the department of Pediatrics

at U.B.C. (which has continued to the present day). In 1986, he became a professor at U.B.C.

From 1989 until 1996, he was the first Scientific Director of the newly established Canadian Bacterial Diseases Network. Under his direction, the network of academic and applied microbiologists and molecular biologists made fundamental discoveries of the mechanisms of **bacterial infection**. Presently, he is a board member of the network. From 1990 until 1993, Hancock was the Chair of the Medical/Scientific Advisory Committee of the Canadian Cystic Fibrosis Foundation. Finally, beginning in 1997, Hancock has been the Director of the Centre for Microbial Diseases and Host Defense Research at UBC.

Hancock has served on the editorial boards of ten international peer-reviewed journals and his expertise in bacterial pathogenesis and antibiotic resistance is utilized in a consultative and directorial role in a myriad of government and industrial settings.

Through his research, Hancock has revealed some of the molecular aspects of the mechanisms by which *Pseudomonas aeruginosa* is able to cause disease or death, particularly in those afflicted with cystic fibrosis. His research has determined the structure of some outer membrane proteins that functions as transport pores. Additionally, he is among the group that has completed the sequencing of the genome of the bacterium. The latter work will lead to further discoveries of genes that are vital in disease processes.

Hancock's best-known research has been the unraveling of what is termed the "self-promoted uptake" of aminoglycoside, polymyxin and cationic **antibiotics** and antimicrobial peptides. This uptake is a major reason for the acquisition of antibiotic resistance by the bacterium, and so will be the target of treatment strategies.

In recognition of his fundamental contributions to bacteriology, Hancock has been the recipient of numerous awards and honors, including the Canadian Society of Microbiologists Award in 1987, the 125th Anniversary of Canada Silver Medal in 1993, inclusion in the *American Men and Women in Science,* 1989–2000, and the MRC Distinguished Scientist Award, 1995–2000.

See also Bacterial adaptation; Infection and resistance

HAND-FOOT-MOUTH DISEASE

Hand-foot-mouth disease is a contagious illness that strikes predominantly infants and children that is characterized by fever, mouth sores, and a rash with blistering. Two types of **viruses** cause the disease. The majority of cases are due to several members of the Coxsackie virus group (subtypes A16, A5, and A10). A type of enterovirus designated as enterovirus 71 also causes the disease, but is of minor importance.

The name of the disease has caused confusion with the well-known hoof and mouth disease. However, hand-foot-mouth disease is entirely different from hoof and mouth disease that strikes cattle, sheep, and swine, causes entirely different symptoms, and which is caused by a different virus.

The disease was initially described and the viral agents determined in 1957.

Hand-foot-mouth disease begins with a general feeling of being unwell. A mild fever, poor appetite, and sore throat leads within a few days to the appearance of sores in the mouth. The blister-like rash develops soon thereafter on the palms of the hands, soles of the feet, on the inside of the mouth, and sometimes on the buttocks. The hands tend to be involved more than the other regions of the body. These symptoms are more inconvenient than threatening to health. Recovery is typically complete within a week or two. Rarely, a stiff neck and back pain reminiscent of **meningitis** can lead to hospitalization. This precaution is prudent, since one of the enteroviruses that causes hand-foot-mouth disease, enterovirus 71, can also cause viral meningitis. During outbreaks of hand-foot-mouth disease, cases of viral meningitis can concurrently appear.

Children fewer than ten years of age are most susceptible. However, the disease can occur in adults as well. In children the fever, which can peak in the range of 103 to 104° F (39.4 to 39.9° C), is a concern. Also, the sores in the mouth can discourage children from eating and drinking. Thus, an important aspect of managing the disease is the maintenance of a sufficient diet.

The disease is contagious and can be spread from person to person by direct contact with nose or throat fluids. There is no geographic restriction on the occurrence of the disease. There is some seasonal distribution, with the majority of cases being reported during the summer and early fall.

Treatment of hand-foot-mouth disease is confined to the relief of the symptoms, and observance of good hygienic practices to minimize the spread of the virus. **Antibiotics** are useless, given the viral nature of the disease. An actual cure, such as a **vaccine**, does not yet exist. Even if specific **immunity** to one episode of the disease has been produced, a subsequent infection with a different subtype of Coxsackie virus can cause another bout of the disease. In this sense, hand-foot-mouth disease is similar to the immune variation that is the hallmark of influenzae viruses.

HAND WASHING • *see* HYGIENE

HANTAVIRUS AND HANTA DISEASE

Hantavirus (family Bunyaviridae, genus Hantavirus) infection is caused by **viruses** that can infect humans with two serious illnesses: hemorrhagic fever with renal syndrome (HFRS), and Hantavirus pulmonary syndrome (HPS).

Hantaviruses are found without causing symptoms within various species of rodents and are passed to humans by exposure to the urine, feces, or saliva of those infected rodents. Infection is commonly associated with disturbing the droppings or nests of rodents within confined spaces. The disturbed particles are inhaled to cause infection. Ten different hantaviruses have been identified as important in humans.

Each is found in specific geographic regions, and therefore is spread by different rodent carriers. Further, each type of virus causes a slightly different form of illness in its human hosts. Hantaan virus is carried by the striped field mouse, and exists in Korea, China, Eastern Russia, and the Balkans. Hantaan virus often causes a severe form of hemorrhagic fever with renal syndrome (HFRS).

Puumula virus is carried by bank voles, and exists in Scandinavia, western Russia, and Europe. Puumula virus causes a milder form of HFRS, usually termed nephropathia epidemica.

Seoul virus is carried by a type of rat called the Norway rat, and exists worldwide, but causes disease almost exclusively in Asia. Seoul virus causes a form of HFRS which is slightly milder than that caused by Hantaan virus, but results in liver complications.

Prospect Hill virus is carried by meadow voles and exists in the United States, but has not been found to cause human disease. Sin Nombre virus, the most predominant strain in the United States, is carried by the deer mouse. This virus was responsible for severe cases of HPS that occurred in the Southwestern United States in 1993. A similar, but genetically distinct strain was responsible for an outbreak of HPS in Argentina in 1996, and was later termed the Andes virus. During the outbreak in Argentina, five of the victims were physicians, three of which were directly responsible for the care of an HPS patient. This, along with additional epidemiologic evidence (such as the low rodent population density in the area affected) suggest that person-to-person transmission was possible during this outbreak, a feature unique to any known hantavirus.

Black Creek Canal virus has been found in Florida. It is predominantly carried by cotton rats. New York virus strain has been documented in New York State. The vectors for this virus appear to be deer mice and white-footed mice. Bayou virus has been reported in Louisiana and Texas and is carried by the marsh rice rat.

Blue River virus has been found in Indiana and Oklahoma and seems to be associated with the white-footed mouse. Monongahela virus, discovered in 2000, has been found in Pennsylvania and is transmitted by the white-footed mouse.

Hantaviruses that produce forms of hemorrhagic fever with renal syndrome (HFRS) cause a classic group of symptoms, including fever, malfunction of the kidneys, and low platelet count. Because platelets are blood cells important in proper clotting, low numbers of circulating platelets can result in spontaneous bleeding, or hemorrhage.

Patients with HFRS have pain in the head, abdomen, and lower back, and may report bloodshot eyes and blurry vision. Tiny pinpoint hemorrhages, called petechiae, may appear on the upper body and the soft palate in the mouth. The patient's face, chest, abdomen, and back often appear flushed and red, as if sunburned. Around day eight of HFRS, kidney involvement results in multiple derangements of the body chemistry. Simultaneously, the hemorrhagic features of the illness begin to cause spontaneous bleeding, as demonstrated by bloody urine, bloody vomit, and in very serious cases, brain hemorrhages with resulting changes in consciousness and shock.

Hantavirus pulmonary syndrome (HPS) develops in four stages. They are: The incubation period. This lasts from one to five weeks from exposure. Here, the patient may exhibit no symptoms.

The prodrome, or warning signs, stage. The patient begins with a fever, muscle aches, backache, and abdominal pain and upset. Sometimes there is vomiting and diarrhea.

The cardiopulmonary stage. The patient slips into this stage rapidly, sometimes within a day or two of initial symptoms; sometimes as long as 10 days later. There is a drop in blood pressure, shock, and leaking of the blood vessels of the lungs, which results in fluid accumulation in the lungs, and subsequent shortness of breath. The fluid accumulation can be so rapid and so severe as to put the patient in respiratory failure within only a few hours. Some patients experience severe abdominal tenderness.

The convalescent stage. If the patient survives the respiratory complications of the previous stage, there is a rapid recovery, usually within a day or two. However, abnormal liver and lung functioning may persist for six months.

The diagnosis of infection by a hantavirus uses serologic techniques. The **ELISA** (**enzyme-linked immunosorbant assay**) is done in a laboratory to identify the presence of specific immune substances (antibodies), which an individual's body would only produce in response to the hantavirus. It is difficult to demonstrate the actual virus in human tissue or to grow cultures of the virus within the laboratory, so the majority of diagnostic tests use indirect means to demonstrate the presence of the virus.

Treatment of hantavirus infections is primarily supportive, because there are no agents available to kill the viruses and interrupt the infection. The diseases caused by hantaviruses are lethal. About 6–15% of people who contract HFRS have died. Almost half of all people who contract HPS will die. It is essential that people living in areas where the hantaviruses exist seek quick medical treatment, should they begin to develop an illness that might be due to a hantavirus. Preventative measures focus on vector control (elimination of rodents), and avoiding rodent infested areas.

See also Epidemics, viral; Epidemiology, tracking diseases with technology; Epidemiology; Hemorrhagic fevers and diseases; Virology

Hazard Analysis and Critical Control Points program (HACCP)

Hazard Analysis and Critical Control Points (HACCP) refers to a system that is established and instituted to monitor all stages of a processing or manufacturing operation to ensure that the final product is not compromised because of microbial **contamination**. Originally, HACCP was devised for the food processing industry. Now, HACCP has expanded to include the manufacture of pharmaceuticals and other products that could be affected by unwanted growth of **microorganisms**.

The global scope of the HACCP program reflects the susceptibility of, for example, a food to contamination from different microorganisms at different stages of the processing pathway. The altered storage conditions, physical state and chemistry of the food, and shipping conditions can select for the growth of different microbes. The absence of microbes as a food enters a plant is no guarantee that the food will remain uncontaminated. The monitoring of points along the production pathway that are deemed critical and susceptible to contamination can reveal problems and spur remediation of the problems.

The concept of HACCP arose in 1959 at the Pillsbury Company. At that time, the company was contracted to provide food products for the United States manned space program. A system of stringent quality control was needed, because astronauts would not have access to medical attention in the event of the development of a food poisoning or intoxication event in space. The concept of a controlled series of checks was born. In 1971, at the United States Conference on Food Protection, the principles of HACCP were formally described. The principles and procedures of a HACCP program have since been formalized by the National Advisory Committee on Microbiological Criteria for Foods.

Then, as now, there are three principles to the program. First is the identification of hazards found all the way from the field to the marketplace, and the determination of the urgency of each hazard in terms of remedy. Second is the formulation of steps to control or prevent the occurrence of those hazards that warrant remediation. Last is the establishment of a system to monitor the points in the manufacturing process that have been deemed either critical to product quality or a point at which contamination could occur.

A critical part of a HACCP program is the writing of what are known as standard operating procedures (or SOPs). A SOP documents exactly how a test or monitoring will be conducted, how often it will be conducted, and both how—and to whom—the results of the test or monitoring will be reported. This ensures that the food or other material that is being guarded from contamination will be treated the same way. In this way, if a problem occurs, there is a standard in place, which allows for a frame of reference in which to properly evaluate the problem.

A properly operating HACCP program is not, of course, a guarantee that no problems will occur. However, the chances of microbial contamination will be reduced, because problems will be noted as they develop (for example, the need to clean a piece of equipment or the accumulation of stagnant water in a pipeline), rather than being confronted by a contamination problem with no warning. Knowledge of the types of contamination problems that can arise can help pinpoint the source of a contamination, and so can minimize the time that a production line is shut down.

An important aspect of a HACCP program is that all remediative procedures that are contemplated must be capable of being routinely performed. If a solution is not easily done, the control step is meaningless. An adjunct to a successful HACCP is the industrial concept of Good Manufacturing Practices (abbreviated GMP). Essentially GMP is a series of quality control measures designed to ensure that a process proceeds as planned.

Another important aspect of a HACCP program is the verification that the program is operating properly. This can involve the use of known strains of **bacteria** to verify that the examination techniques being used to monitor a process do indeed detect the bacteria. Such tests should be performed regularly (daily, weekly, monthly) and the results should be documented.

HACCP programs involving microorganisms typically are revised as more becomes known about the microbe of interest. As further information is learned of, for example, the microbe's habitat, growth conditions, and environmental niches, more monitoring or the use of additional examination techniques may need to be incorporated into the HACCP program.

See also Disinfection and disinfectants; Food safety

HAZEN, ELIZABETH (1885-1975)

American microbiologist

Elizabeth Hazen, through a long-distance collaboration with her colleague Rachel Brown, developed the first non-toxic drug treatment for fungal infections in humans.

Hazen was born in Rich, Mississippi, on August 24, 1885, and raised by relatives in Lula, Mississippi, after the death of her parents. Hazen attended the public schools of Coahoma County, Mississippi, and earned a B.S. from the State College for Women, now Mississippi University for Women. She began teaching high school science and continued her own education during summers at the University of Tennessee and University of Virginia.

In 1916, Hazen began studying bacteriology at Columbia University, where she earned an M.A. the following year. World War I provided some opportunities for women scientists, and Hazen served in the Army diagnostic laboratories and subsequently in the facilities of a West Virginia hospital. Following the war, she returned to Columbia University to pursue a doctorate in microbiology, which she earned in 1927 at age 42.

After a four-year stint at Columbia University as an instructor, Hazen joined the Division of Laboratories and Research of the New York State Department of Health. She was assigned to special problems of bacterial diagnosis and spent the next few years researching bacterial diseases. She investigated an outbreak of **anthrax**, tracing it to a brush factory in Westchester County. Hazen discovered unknown sources of **tularemia** in New York and was the first to identify imported canned seafood that had spoiled as the cause of type E toxin deaths.

Her discoveries led Hazen to try to better understand mycotic (fungal) diseases. In 1944, she was given the responsibility of investigating such diseases, and she acquired cultures of **fungi** from local laboratories and specialized collections. Although Hazen was learning more about mycotic diseases, fungal infections continued to spread in epidemic proportions among school children in New York City. In addi-

tion to **pneumonia**, many other fungal diseases caused widespread ailments, such as moniliasis (**thrush**), a mouth condition that makes swallowing painful. Despite personal health problems and stressful working conditions, Hazen persevered.

In the mid-1940s, she teamed up with Rachel Brown (1898–1980), a chemist at the Albany laboratory who prepared extracts from the cultures sent by Hazen. In the fall of 1950, Hazen and Brown announced at a National Academy of Sciences meeting that they had successfully produced two antifungal agents from an antibiotic. This led to their development of Nystatin, the first fungicide safe for treating humans. Nystatin was immediately used nationwide, earning $135,000 in its first year.

Nystatin, which is still sold as a medication today under various trade names, turned out to be an extremely versatile substance. In addition to curing serious fungal infections of the skin and digestive system, it can also combat Dutch Elm disease in trees and even restore artwork damaged by water and **mold**. Remarkably, Hazen and Brown chose not to accept any royalties from the patent rights for Nystatin. Instead, they established a foundation to support advances in science. The donated royalties totaled more than $13 million by the time the patent expired. Hazen died on June 24, 1975.

See also Candidiasis; Fungicide; Yeast, infectious

HEAT SHOCK RESPONSE

The heat shock response occurs in virtually all organisms, including **bacteria**. The response occurs when an environmental stress is imposed on the organism. The name of the response comes from its discovery following the application of a mild heat stress, only 5 to 10° C higher than the usual preferred growth temperature. However, the response, which can be more correctly thought of as an adaptive response, consisting of a temporary alteration in the **metabolism** of the organism, occurs in response to more than just excessive heat.

Hallmarks of the heat shock response are its rapid onset and short-term nature. The response is an emergency coping type of reaction to a conditions that is perceived by the organism as being threatening. The response is not a long-term commitment, such as the formation of a spore by a bacterium (although some proteins that are stimulated in the heat shock response of *Bacillus subtilis* also function in the formation of spores by the bacteria). Rather, a heat shock response allows the organism to cope and then to quickly resume normal function.

The alteration in the chromosome of the fruit fly *Drosophila* flowing an elevation in the temperature was reported in 1962. At the time and for some years thereafter, the observation was regarded as an interesting curiosity that was relevant only to the fruit fly. However, it is unequivocally clear that the genes encoding the responsive proteins and the structure of the proteins themselves are highly conserved in many species. For example, three heat shock proteins that were discovered in bacteria, which have been dubbed Hsp70, Hsp10, and Hsp60 are highly conserved in a large number of bacteria and in eukaryotic organisms.

Heat shock proteins are induced in large quantities in response to factors including nutrient depletion, addition of alcohols such as ethanol, change in the sodium concentration, presence of heavy metals, fever, interaction with cells of a host, and the presence of virus. The proteins are produced in non-stress conditions. But typically their quantities are much less. In non-stress conditions they function in the normal metabolic events within the cell.

The heat shock response in bacteria involves the elevated production of more than 20 proteins whose functions are varied. For example, some of the proteins degrade other proteins (proteases), while other proteins help transport molecules from one place to another while preserving their structure (these are known as **chaperones**). These induced proteins act to overcome the changes that would prove destructive to other proteins in the bacterium. By preventing protein alteration and destruction, the heat shock response ensure that the bacterium will be capable of normal function once the stress is removed.

Some bacteria also utilize the heat shock response to promote infection of host cells or tissue, or to survive within host cells. Examples of bacteria include ***Escherichia coli***, *Legionella pneumophila*, and *Listeria monocytogenes*. Furthermore, the alteration in structure of bacteria can involve heat shock proteins. Examples include *Bacillus subtilis* spore formation and the formation of the so-called fruiting bodies by myxobacteria.

The principle trigger for the heat shock response in bacteria is at the level of **transcription,** where the genetic material, **DNA** (**deoxyribonucleic acid**), is used to manufacture **ribonucleic acid**. In *Escherichia coli* the response is controlled by what has been called a sigma factor. The sigma factor is capable of binding to various regions of the DNA that stimulate the transcription of the particular **gene** under their control. In other words, the single sigma factor is capable of stimulating the expression of multiple genes. The sigma factor is under a tight and complex control, which normally restricts its activity but leaves the factor primed for action. This is the reason for the rapid nature of the heat shock response.

Two other heat shock response controls in bacteria operate after the proteins are produced. These controls aid in maintaining the proteins for a bit longer than if they were produced under non-heat shock conditions. By preserving the structure and functions of the heat shock proteins, their activity is allowed to persist. But, once again, the protein activity does not last indefinitely, which allows the heat shock response to be rapidly "turned off."

The observations that bacterial heat shock proteins can be vital for the establishment of infections has made the heat shock response the subject of much study, with the aim of circumventing the response or devising vaccines that protect host cells.

See also Bacterial adaptation; Microbial genetics

HELICOBACTERIOSIS

Helicobacteriosis is an infection of the gastrointestinal tract that is caused by the Gram-negative, spiral-shaped bacterium called *Helicobacter pylori*. Ulcers on the lining of the stomach and upper intestinal tract characterize the malady. The ulceration may be a prelude to the development of cancer of the stomach.

Helicobacteriosis is established following the colonization of the stomach by *Helicobacter pylori*. How the **bacteria** are transmitted to a person is still unclear. The prevalence of the infection in overcrowded environments, especially where children are present, indicate that person-to-person transmission is most likely, and that personal **hygiene** plays a role in transmission. Following transmission, the bacterium is able to persist in the extremely acidic environment of the stomach by burrowing under the mucous overlay of the stomach epithelial cells, and because the bacteria produce an enzyme called urease. The enzyme is able to degrade the gastric acid in the stomach.

Helicobacteriosis invariably becomes chronic. Then, the infection can also be referred to as chronic gastritis. The infection can become chronic because initially the infection produces little or no symptoms. Thus, the **immune system** is not alerted to response to the infection, which provides an opportunity for the bacterial population to become more tenaciously established.

In about 15% of those who become infected, ulcers develop in the stomach or in a region of the upper intestine called the duodenum. The resulting burning feeling caused by increased secretion of acid is relieved by over-the-counter antacids, which can further dissuade people from seeking a physician's care for the malady. If the infection is diagnosed and the bacteria eliminated by antibiotic therapy, the elevated production of acid stops. But, in the absence of treatment, the painful ulcers will recur. Why only fifteen per cent of those who have infections develop ulcers while the other 85% of infected individuals do not is not clear.

Moreover, the molecular basis for the establishment of the ulcers is also still not clear. There has been some indication of toxin involvement. *Helicobacter pylori* produces a toxin called VacA and a protein called CagA.

More ominously, epidemiologic evidence strongly indicates that *Helicobacter pylori* stomach infections are associated with the development of various types of stomach cancers. For examples, the bacterial infection is nine times more common in those patients who are diagnosed with cancer of the stomach, and seven times more common in those people who have a tumor of the lymphatic tissue. Studies have demonstrated that even in these advanced cases, the elimination of *Helicobacter pylori* can produce a shrinking of the tumors.

Helicobacteriosis can be detected in three ways. The first way is by obtaining a sample of stomach tissue. Culturing of the stomach contents on growth media that selects for the growth of *Helicobacter pylori* over other bacteria is used to isolate the organism. If the region of the stomach where tissue is obtained is free from bacteria, then an infection can be missed.

A second way of detecting the presence of the bacteria is by a breath test. Breathing on a specially prepared support can detect the presence of the urease enzyme that is produced by *Helicobacter pylori*. Since this enzyme is not commonly produced, the detection of the enzyme is a strong indication of the presence of living bacteria. However, in the absence of the actual isolation of the bacteria, the breath test cannot be absolutely diagnostic.

Finally, antibodies produced in response to a *Helicobacter pylori* infection can be detected by a blood test.

Once detected, the bacterial infection does respond to antibiotic therapy. Elimination of the infection relieves the symptoms of helicobacteriosis in 80% of those people who are infected.

The discovery that helicobacteriosis has a bacterial origin and the relief of the symptoms upon bacterial eradication has reinforced the validity of a theory that proposes that many chronic and autoimmune diseases, such as certain types of heart disease and rheumatoid arthritis, are caused by an infection by bacteria or other microbe.

See also Bacteria and bacterial infection; Microbial flora of the stomach and gastrointestinal tract

HEMAGGLUTININ (HA) AND NEURAMINIDASE (NA)

Hemagglutinin (designated as HA) and neuraminidase (designated as NA) are glycoproteins. Hemagglutinin and neuraminidase protrude from the outer surface of the **influenza** virus and neuraminidase is a constituent of the enveloping membrane that surrounds the viral contents. A glycoprotein is a protein that contains a short chain of sugar as part of its structure. The hemagglutinin and neuraminidase glycoproteins are important in the ability of the virus to cause influenza.

A typical influenza virus particle contains some 500 molecules of hemagglutinin and 100 molecules of neuraminidase. These are studded over the surface of the virus.

The illness caused by the influenza virus can be devastating. For example, in 1918 a new genetic variant of the virus swept around the world and in just over a year over 20 million people succumbed to the influenza. The variation was due to alterations in both the hemagglutinin and neuraminidase components of the virus. Further antigenic variations of these molecules produced a virus that, at least for some time, was not recognized by the **immune system**. The result was localized outbreaks or worldwide outbreaks in 1957, 1962, 1964, 1976, and 1978.

Hemagglutinin derives its name from its activity. The glycoprotein confers upon the virus the ability to agglutinate, or clump together, red blood cells. The aggregation compromises the function of the red blood cells. The hemagglutinin glycoprotein also functions in the binding of the virus to cells, via the recognition of a chemical structure on the cells surface called sialic acid. The binding of hemagglutinin to sialic acid compounds on the surface of cells is the initial event in the association of the virus with human epithelial cells. These two activities associated with hemagglutinin are important activi-

ties in the infectious ability of the virus. Indeed, hemagglutinin is the major virulence (disease-causing) factor of the influenza virus.

There are three distinct haemagglutinins important in human infections that are encoded by genes in the virus. These are designated as H1, H2, and H3. Animal influenza **viruses** contain nine additional types of hemagglutinin.

Neuraminidase is the common name for acetyl-neuraminyl hydrolase. The glycoprotein compound is an enzyme. The enzyme removes residues called N-Acetyl-neuraminic acid from chains of sugars and from other glycoproteins. The disruption of the neuraminic acid residues allows the virus to both pass out of the human epithelial cells in which it is replicating, and enter new cells to initiate a new round of viral replication. The activity of neuraminidase disrupts the mucous fluid that is present in the respiratory tract. Also, possession of neuraminidase keeps the viruses from aggregating with other virus particles. The result of these activities is to ease the spread of the virus through the respiratory tract.

Two different species of neuraminidase, designated N1 and N2, are important in human infections, while seven additional species are important in animal influenza viruses.

Inhibitors of neuraminidase have been developed in an effort to thwart the viral infection. The inhibitors are structurally similar to the silica acid on the surface of human epithelial cells. The rational is that the virus will bind to the inhibitor rather than to the human cells, and the inhibitor-viral complex can be removed from the body.

Hemagglutinin and neuraminidase are used in the designation of the different antigenic types of the influenza virus that have and continue to appear. For example, Influenza A/Taiwan/86/H1N1 is an influenza A strain of the H1 hemagglutinin type and N1 neuraminidase type that was first isolated in Taiwan in 1986.

Both hemagglutinin and neuraminidase tend to undergo what is termed antigenic drift, which is a slight but frequent change in the antigenic character. The slight change is still usually enough to thwart the recognition capabilities of the immune system. Hence, annual vaccinations are necessary to minimize the chance of acquiring an influenza infection. A major antigenic change in one or both of the glycoproteins, as happened in the 1918 virus, is termed antigenic shift.

See also Flu, the great flu epidemic of 1918; Mutations and mutagenesis

HEMOLYSIS AND HEMOLYTIC REACTIONS

• *see* BLOOD AGAR, HEMOLYSIS, AND HEMOLYTIC REACTIONS

HEMOLYTIC REACTIONS

• *see* BLOOD AGAR, HEMOLYSIS, AND HEMOLYTIC REACTIONS

HEMORRHAGIC FEVERS AND DISEASES

Hemorrhagic diseases are caused by infection with **viruses** or **bacteria**. As the name implies, a hallmark of a hemorrhagic disease is copious bleeding. The onset of a hemorrhagic fever or disease can lead to relatively mild symptoms that clear up within a short time. However, hemorrhagic diseases are most recognized because of the ferocity and lethality of their symptoms as well as the speed at which they render a person extremely ill.

Virtually all the hemorrhagic diseases of microbiological origin that arise with any frequency are caused by viruses. The various viral diseases are also known as viral hemorrhagic fevers. Bacterial infections that lead to hemorrhagic fever are rare. One example is a bacterium known as scrub **typhus**.

The viruses that cause hemorrhagic diseases are members of four groups. These are the arenaviruses, filoviruses, bunyaviruses, and the flaviviruses. Arenaviruses are the cause of Argentine hemorrhagic fever, Bolivian hemorrhagic fever, Sabia-associated hemorrhagic fever, Lassa fever, Lymphocytic choriomeningitis, and Venezuelan hemorrhagic fever. The Bunyavirus group causes Crimean-Congo hemorrhagic fever, Rift Valley fever, and Hantavirus pulmonary syndrome. Filoviruses are the cause of Ebola hemorrhagic fever and Marburg hemorrhagic fever. Lastly, the Flaviviruses cause tick-borne encephalitis, **yellow fever**, Dengue hemorrhagic fever, Kyasanur Forest disease, and Omsk hemorrhagic fever.

While the viruses in the groups display differences in structure and severity of the symptoms they can cause, there are some features that are shared by all the viruses. For instance, all the hemorrhagic viruses contain **ribonucleic acid** as their genetic material. The nucleic acid is contained within a so-called envelope that is typically made of lipid. Additionally, all the viruses require a host in which to live. The animal or insect that serves as the host is also called the natural reservoir of the particular virus. This natural reservoir does not include humans. Infection of humans occurs only incidentally upon contact with the natural reservoir.

Hemorrhagic diseases can be devastating for the victim. The symptoms can progress from mild to catastrophic in only hours. As a result, an outbreak of hemorrhagic disease tends to be self-limiting in a short time. In some cases, this is because the high death rate of those who are infected literally leaves the virus with no host to infect. Often the outbreak fades away as quickly as it appeared.

Hemorrhagic fever related illnesses appear in a geographical area where the natural reservoir and human are both present. If the contact between the two species is close enough, then the disease causing microorganism may be able to pass from the species that is the natural reservoir to the human.

Although little is still clear about the state of the microbes in their natural hosts, it is reasonably clear now that the viruses do not damage these hosts as much as they do a human who acquires the **microorganisms**. Clarifying the reasons for the resistance of the natural host to the infections would be helpful in finding an effective treatment for human hemorrhagic diseases.

The speed at which hemorrhagic fevers appear and end in human populations, combined with their frequent occurrence in relatively isolated areas of the globe has made detailed study difficult. Even though some of the diseases, such as Argentine hemorrhagic fever, have been known for almost 50 years, knowledge of the molecular basis of the disease is lacking. For example, while it is apparent that some hemorrhagic viruses can be transmitted through the air as aerosols, the pathway of infection once the microorganism has been inhaled is still largely unknown.

The transmission of hemorrhagic viruses from the animal reservoir to humans makes the viruses the quintessential zoonotic disease. For some of the viruses the host has been determined. Hosts include the cotton rat, deer mouse, house mouse, arthropod ticks, and mosquitoes. However, for other viruses, such as the Ebola and Marburg viruses, the natural host still remains undetermined. Outbreaks with the Ebola and Marburg viruses have involved transfer of the virus to human via primates. But, whether the primate is the natural host, or acquired the virus as the result of contact with the true natural host is not clear.

Another fairly common feature of hemorrhagic diseases is that once humans are infected with the agent of the disease, human-to-human transmission can occur. Often this transmission can be via body fluids that accidently contact a person who is offering care to the afflicted person.

Hemorrhagic diseases typically begin with a fever, a feeling of tiredness, aching of muscles. These symptoms may not progress further, and recovery may occur within a short time. However, damage that is more serious can occur, which is characterized by copious bleeding, often from orifices such as the mouth, eyes, and ears. More seriously, internal bleeding also occurs, as organs are attacked by the infection. Death can result, usually not from loss of blood, but from nervous system failure, coma, or seizures.

Hemorrhagic diseases are difficult to treat. Vaccines are available to only yellow fever and Argentine hemorrhagic fever. For the remaining diseases, the best policy is to curb the potential for human interaction with the natural reservoir of the microbe. For example, in the case of hantavirus pulmonary syndrome, control of the rodent population, especially after a rainy season when numbers naturally increase, is a wise course. Insect vectors are controlled by a concerted campaign of spraying and observance of precautionary measures (e.g., use of insect repellent, proper clothing, insect netting over sleeping areas, etc.).

See also Public health, current issues; Viruses and response to viral infection

HEPADNAVIRUSES

Hepadnaviridae is a family of hepadnaviruses comprised by two genera, *Avihepadnavirus* and the *Orthohepadnavirus*. Hepadnaviruses have partially double strands **DNA** (partial dsDNA virion) and they replicate their genome in the host cells using reverse transcriptase and are therefore, termed **retroviruses**. Their virion DNA, invades the hepatocytes (i.e., liver cells) of vertebrates, which are their natural hosts. When hepadna retroviruses invade a cell, a complete viral dsDNA is made before its random integration in one of the host's **chromosomes**, and is then transcribed into an intermediate messenger **RNA** (mRNA) into the hosts' **nucleus**. The viral mRNA then leaves the nucleus and undergoes reverse transcriptase, mediated by a viral reverse transcriptase enzyme that transcribes complementary strands of complementary dsDNA in the cell cytosol, thus forming new partial dsDNA virions.

Orthohepadnavirus is the pathogenic agent that causes chronic **hepatitis** (Hepatitis type B) in mammals, which may eventually lead to either cirrhosis or liver cancer if not detected and treated. Hepatitis B Virus or HBV, the prototype member of the family Hepadnaviridae, is transmitted by both infected blood (blood transfusions, grafts) and body fluids (usually through sexual relations with infected partners). HBV comprises several viral species that also infect the liver cells of orangutans, dogs, and other mammalians besides man. Vaccines for both human Hepatitis B and several forms of animal Hepatitis B (lions, cats, dogs) are available as a form of disease prevention. All Hepadnaviridae **viruses** have a high affinity with liver cells (hepatotropy) and the viruses of the genus *Avihepadnavirus,* also known as avian hepadnaviruses, have as targets the liver of birds, such as storks, ducks, herons, etc.

See also Animal models of infections; Antiviral drugs; Hepatitis, hepatitis viruses and tests; Interferons; Virology

HEPATITIS AND HEPATITIS VIRUSES

Hepatitis is **inflammation** of the liver, a potentially life-threatening disease most frequently caused by viral infections but which may also result from liver damage caused by toxic substances such as alcohol and certain drugs. Hepatitis **viruses** identified to date occur in six major types: hepatitis A HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis D (HDV), and hepatitis E (HEV) and hepatitis G (HGV). All types are potentially serious and, because clinical symptoms are similar, positive identification of the infecting strain is possible only through serologic testing (analyzing the clear, fluid portion of the blood). Symptoms may include a generalized feeling of listlessness and fatigue, perhaps including mental depression, nausea, vomiting, lack of appetite, dark urine and pale feces, jaundice (yellowing of the skin), pain in the upper right portion of the abdomen (where the liver is located), and enlargement of both the liver and the spleen. Severe cases of some types of hepatitis can lead to scarring and fibrosis of the liver (cirrhosis), and even to cancer of the liver.

Epidemics of liver disease were recorded as long ago as Hippocrates' time and, despite major advances in diagnosis and prevention methods over the past two decades, viral hepatitis remains one of the most serious global health problems facing humans today.

The incidence and spread of HAV is directly related to poor personal and social **hygiene** and is a serious problem not only in developing countries where sanitation and water

purification standards are poor, but also in developed, industrialized nations, including the United States, where it accounts for 30% of all incidences of clinical hepatitis. Except in one to four percent of cases where sudden liver failure may result in death, chronic liver disease and serious liver damage very rarely develop, and "chronic carrier state," in which infected people with no visible symptoms harbor the virus and transfer the disease to non-infected individuals, never occurs. Also, reinfection seldom develops in recovered HAV patients because the body eventually develops antibodies, cells which provide a natural **immunity** to the specific virus attacking the host. Although HAV is self-limiting (after time, ends as a result of its own progress), there is as yet no effective treatment once it is contracted.

Apart from the symptoms described above, HAV commonly produces a medium-grade fever, diarrhea, headaches, and muscle pain. The primary route of HAV transmission is fecal-oral through ingestion of water contaminated with raw sewage, raw or undercooked shell-fish grown in contaminated water, food contaminated by infected food handlers, and close physical contact with an infected person. Heterosexual and homosexual activities with multiple partners, travel from countries with low incidences to countries with high rates of infected population, and, less frequently, blood transfusions and intravenous drug use also spread infection.

During the infectious stage, large numbers of viruses are eliminated with the stool. Although HAV infection occurs in all age groups, high rates of disease transmission occur in day-care centers and nursery schools where children are not yet toilet trained or able to wash their hands thoroughly after defecating. The disease may then be transmitted to day-care workers and carried home to parents and siblings. In areas of the world where living quarters are extremely crowded and many people live in unhygienic conditions, large outbreaks of HAV threaten people of all ages. Because during the viruses' incubation period—from 14 to 49 days—no symptoms are observable, and because symptoms seldom develop in young children, particularly those under the age of two, the disease is often unknowingly but readily transmitted before infected people can be isolated.

A **vaccine** against HAV is available. It appears to provide good protection, if the first **immunization** has been received at least four weeks prior to exposure. For adults, two immunizations about 6 months apart are recommended; for children, three immunizations are necessary (two a month apart, and the third six months later). High-risk groups who should receive HAV vaccine include child care workers, military personnel, Alaskan natives, frequent travelers to HAV endemic areas, laboratory technicians where HAV is handled, people who work with primates. The immunization lasts for 20 years.

If someone who is unimmunized is exposed to HAV, or if a traveler cannot wait four weeks prior to departure for an HAV endemic area, then immune globulin may be utilized to avoid infection. Immune globulin is a naturally occurring substance harvested from the plasma in human blood, then injected into an individual exposed to the HAV. Immune globulin prevents disease development in 80–90% of cases in clinical trials. It also seems to be effective in reducing the number of cases normally expected after outbreaks in schools and other institutions. As yet, the most effective control mechanisms are public education regarding the importance of improved personal hygiene, which in many instances is as simple as washing hands thoroughly after using the toilet and before handing food, and concerted worldwide efforts to purify water supplies (including rivers and oceans) and improve sanitation methods.

Vaccination against Hepatitis B virus.

Acute HBV is currently the greatest cause of viral hepatitis throughout the world. **World Health Organization** figures released in 1992 indicate that as many as 350 million people worldwide carry the highly infectious HBV. Because of its severity and often lengthy duration, 40% of those carriers—possibly as many as two million per year—will eventually die from resultant liver cancer or cirrhosis. HBV-related liver cancer deaths are second only to tobacco-related deaths worldwide. Infected children who survive into adulthood may suffer for years from the damage caused to the liver. In the United States alone, as many as 300,000 people become infected with HBV every year, medical costs amount to more than $1 million per day, and the death rate over the last 15 or so years has more than doubled in the United States and Canada.

If **serology** (blood) tests detect the presence of HBV six months or more from time of initial diagnosis, the virus is then termed "chronic." Chronic persistent hepatitis may develop following a severe episode of acute HBV. Within a year or two, however, this type usually runs its course and the patient recovers without serious liver damage. Chronic active hepatitis also may follow a severe attack of acute HBV infection, or it may simply develop almost unnoticed. Unlike persistent hepatitis, the chronic active type usually continues until fatal liver damage occurs. In long-term studies of 17 patients with chronic active hepatitis, 70% developed cirrhosis of the liver within two to five years.

Symptoms are similar to those manifested by HAV and may include weight loss, muscle aches, headaches, flu-like symptoms, mild temperature elevation, and constipation or diarrhea. By the time jaundice appears, the patient may feel somewhat better overall but the urine becomes dark, stools light or yellowish, the liver and possibly the spleen enlarged and painful, and fluid may accumulate around the abdominal area. Early in the disease's life, however, symptoms may be very slight or even virtually nonexistent, particularly in children, facilitating infection of others before isolation is implemented.

The incubation period for HBV varies widely—anywhere from four weeks to six months. Primary routes of transmission are blood or blood product transfusion; body fluids such as semen, blood, and saliva (including a bite by an infected human) organ and/or tissue transplants; contaminated needles and syringes in hospitals or clinical settings; contaminated needles or syringes in illegal intravenous drug use; and vertical transmission—from mother to baby during pregnancy, birth, or after birth through breast milk. Even though they may not develop symptoms of the disease during childhood, and will remain healthy, almost all infected newborns become chronic carriers, capable of spreading the disease. Many of these infected yet apparently healthy children, particularly the males, will develop cirrhosis and liver cancer in adulthood. Where the incidence of the disease is relatively low, the primary mode of transmission appears to be sexual and strongly related to multiple sex partners, particularly in homosexual men. In locations where disease prevalence is high, the most common form of transmission is from mother to infant.

Controlling HBV infection is an overwhelming task. In spite of the development of safe and effective vaccines capable of preventing HBV in uninfected individuals, and regardless of programs designed to vaccinate adults in high-risk categories such as male homosexuals, prostitutes, intravenous drug users, health-care workers, and families of people known to be carriers, the disease still remains relatively unchecked, particularly in developing countries.

Although effective vaccines have been available since the mid-1980s, the cost of mass immunization world-wide, and particularly in developing countries, was initially prohibitive, while immunizing high-risk adult populations did little to halt the spread of infection. Authorities now believe the most effective disease control method will be immunization of all babies within the first weeks following birth. Concerted efforts of researchers and health authorities worldwide, including the foundation in 1986 of an International Task Force for Hepatitis B Immunization are investigating various avenues for providing cost-effective, mass **vaccination** programs. These include incorporating HBV vaccination into the existing Expanded Program of Immunization controlled by the World Health Organization. Methods of cost containment, storing the vaccine, and distribution to midwives in remote villages (60% of the world's births occur at home), have been designed and are continually being refined to ultimately attain the goal of universal infant immunization. This will not only drastically decrease the number of babies infected through vertical transmission (which constitutes 40% of all HBV transmission in Asia), preventing them from becoming adult carriers, it also provides immunity throughout adulthood.

Finding an effective treatment for those infected with HBV presents a major challenge to researchers—a challenge equal to that posed by any other disease which still remains unconquered. And HBV may present yet another challenge: mutant forms of the virus seem to be developing in resistance to the current vaccines, thus finding a way to survive, replicate and continue its devastating course. Necessary measures in disease control include: education programs aimed at health care workers to prevent accidental HBV transfer—from an infected patient to an uninfected patient, or to themselves; strict controls over testing of blood, blood products, organs, and tissue prior to transfusion or transplantation; and the "passive" immunization with immunoglobulin containing HBV antibodies as soon as possible after exposure to the active virus. Treatment with Interferon and new drug, Lamivudine, have shown positive results in managing HBV.

Relatively recently discovered hepatitis viruses, often called non-A, non-B hepatitis, exist in more than 100 million carriers worldwide, with 175,000 new cases developing each year in the U.S. and Europe.

Not until 1990 were serology tests available to identify the hepatitis C virus (HCV). Research since then has determined that HCV is distributed globally and, like HBV, is implicated in both acute and chronic hepatitis, as well as liver cancer and cirrhosis. Eighty-five percent of all transfusion-related hepatitis is caused by HCV, and mother-baby and sexual transmission are also thought to spread the disease. Symptoms are similar but usually less severe than HBV; however, it results in higher rates of chronic infection and liver disease.

Control and prevention of HCV is a serious problem. First, infected people may show no overt symptoms and the likelihood that infection will become chronic means that many unsuspecting carriers will transmit the disease. Second, HCV infection does not appear to stimulate the development of antibodies, which not only means infected people often become reinfected, it creates a major challenge in the development of an effective vaccine. Third, HCV exists in the same general high-risk populations as does HBV. Combined, these factors make reducing the spread of infection extremely difficult. On a positive note, the development of accurate blood screening for HCV has almost completely eliminated transfusion-related spread of hepatitis in developed countries. Immunoglobulin injections do not protect people who have been exposed to HCV; the search is on for an adequate immunization, although this effort is hampered by characteristics of HCV, which

include rapid mutation of the virus. Treatment with interferon remains the most effective measure in managing the long term effects of HCV.

Undiscovered until 1980, Hepatitis E virus (HEV) is thought to transmit in a similar fashion to HAV. HEV is most prevalent in India, Asia, Africa, and Central America. Contaminated water supplies, conditions that predispose to poor hygiene (as in developing countries), and travel to developing countries all contribute to the spread of HEV. Symptoms are similar to other hepatitis viruses and, like HAV, it is usually self-limiting, does not develop into the chronic stage, and seldom causes fatal liver damage. It does seem, however, that a higher percentage of pregnant women (from 10–20%) die from HEV than from HAV.

Research into the virus was slow because of the limited amounts which could be isolated and collected from both naturally infected humans and experimentally infected primates. Recently, successful genetic **cloning** (artificial duplication of genes) is greatly enhancing research efforts. Surprisingly, research found that antibodies exist in between one to five percent of people who have never been infected with hepatitis. Until an effective vaccine is developed, sanitation remains the most important factor in preventing the spread of HEV.

Because it is a "defective" virus requiring "coinfection" with HBV in order to live and reproduce, HDV alone poses no threat in the spread of viral hepatitis. It also poses no threat to people vaccinated against HBV. However, when this extremely infectious and potent virus is contracted by unsuspecting carriers of HBV, rapidly developing chronic and even fatal hepatitis often follows. The coexistent requirements of HDV as yet remain unclear. Research into development of an effective vaccine is ongoing, and genetic cloning may aid in this effort.

Little is currently known about a relatively recently discovered hepatitis virus, G. HGV appears to be passed through contaminated blood, as is HCV. In fact, many infections with HVG occur in people already infected with HCV. HGV, however, does not seem to change the disease course in people infected with both HCV and HGV. In cases of isolated HGV infection, little liver injury is noted, and there does not appear to be a risk of chronic liver injury. Much more information must be sought about this particular hepatitis virus, and its risks.

See also Epidemics, viral; Interferon actions; Public health, current issues; Viruses and responses to viral infection

HEPATITIS, AUTOIMMUNE • *see* AUTOIMMUNITY AND AUTOIMMUNE DISORDERS

HERPES AND HERPES VIRUS

Herpes is a name given to a common viral infection. The infection can occur in the mouth and in the genitals.

The two forms of herpes are caused by two forms of a herpes virus. Both forms are called herpes simplex virus. Oral herpes is generally caused by herpes simplex type 1 (that is typically shortened to HSV–1). It is also known as human herpes virus 1 (HHV1). Genital herpes is generally caused by herpes simplex type 2 (shortened to HSV–2, which has also been called human herpes virus 2, or HHV2). However, HSV–1 can cause genital herpes and HSV–2 can cause oral herpes.

Rash due to Herpes zoster virus.

There are eight herpes virus types known in humans. HSV–1 (HHV1) and HSV-2 (HHV2) are the forms associated with oral and genital herpes. Human herpes virus 3 is also known as **varicella zoster virus**, and is the cause of chickenpox. HHV4 is the official name of **Epstein-Barr virus**, the major cause of infectious **mononucleosis**. HHV5 is also known as cytomegalovirus. It can cause mononucleosis, **hepatitis** in newborns, and complications in **AIDS** patients. HHV6 causes roseola in children and fever-associated seizures in infants. HHV7 has not yet been associated with any disease, and appears to be present in almost all people. Infection with HHV7 likely occurs early in life. Finally, HHV8 contributes to Kaposi's sarcoma, a relatively rare cancer that predominantly afflicts AIDS patients whose immune systems are failing.

Herpes simplex virus types 1 and 2 appear identical when examined using the high magnification power of the **electron microscope**. Both types are icosahedral in shape; that is, their surface consists of twenty equal-sized and equilateral triangles.

The oral form of herpes is manifest as cold sores or so-called fever blisters, and is common in young children. The virus can be passed from person to person very easily. Only a brief contact is needed for transmission. Cold sores are innocuous in children and adults. However, they can be a very serious health threat in newborns.

The genital form can be apparent as genital sores. These appear as clustered blistery-appearing sores on the vagina, vulva, cervix, penis, buttocks, or the anus. Pain, itching, and a burning feeling during urination can accompany the sores. In more severe cases, the lymph glands can be swollen, with a number of flu-like symptoms evident. These symptoms of what is referred to as primary herpes persist for several weeks then disappear. They can return, usually to a lesser extent, in anywhere from a few weeks to years later. Others who are infected may not display any symptoms whatsoever. Diagnosis of infection in asymptomatic people can still be made, based on the detection of viral antibodies in the blood.

Herpes affects some 80 million people in the United States alone, with one in six of these people having genital herpes. Herpes is spread by human contact. Typically this involves kissing, touching, or sexual contact. Typically, a person is contagious when he or she has open sores. Because of this, contact with others can be minimized when sores are present, thus minimizing the chance of spread. However, it has been proven that genital herpes can be spread even when no symptoms or sores are evident. The chance of this happening is about 10 percent. The spread of herpes via wet toilet seats and the like is now considered to be unlikely.

Studies have shown that the chances of pregnant women passing either herpes simplex virus to the developing fetus are rare. Transfer can occur during childbirth. If open sores are evident at this time, a caesarean section may be considered to avoid the chance of infection.

Herpes simplex virus replicates insides cells of the host. An association between a virus particle and the surface of the host cell starts this process. The host cell is typically that in nerves. This association is specific, involving the recognition of a host surface molecule. Another viral protein then associates with several of the host cell molecules that are collectively termed the herpes virus entry mediators. This second association leads to the fusion of the host and the viral membranes. The contents of the virus can then be emptied into the host cell.

Once in the host cell, the viral deoxyribonucleic acid genetic material somehow enters the **nucleus**. The viral **DNA** is then replicated using the **transcription** machinery of the host. The viral transcription process occurs immediately with certain stretches of the viral DNA and a bit later with other stretches of the DNA. The early **gene** products participate in the replication of the later regions of the viral DNA.

New virus particles can be produced very soon after infection. Or, alternatively, the infection may become what is described as latent. In a latent infection, no viral particles are produced. Viral DNA continues to be replicated along with host DNA until such time as a signal stimulates the transcription of viral genes that are involved in the assembly of new virus particles. Stress, surgery, menstruation, and **skin infections** such as sunburn are known to be signals, although the molecular nature of these stimuli is unclear.

Recurrence of symptoms can be more frequent with people whose immune systems are compromised, such as those with leukemia or acquired **immunodeficiency** syndrome (AIDS). Currently there is no cure for herpes. Physicians can prescribe one of three medications to treat genital herpes. These are acyclovir, famciclovir, and valacyclovir. With or without medication, in general the recurrences become fewer with the passage of time, often ending after five to six years.

Despite this fading of symptoms, the herpes simplex **viruses** can be debilitating aside from their direct affects. They can deplete the body's immune resources, leaving someone more vulnerable to infection by another microbial agent.

See also Latent viruses and diseases; Virus replication

HERSHEY, ALFRED DAY (1908-1997)

American microbiologist

By seeking to understand the reproduction of **viruses**, Alfred Day Hershey made important discoveries about the nature of **deoxyribonucleic acid** (**DNA**) and laid the groundwork for modern **molecular genetics**. Highly regarded as an experimental scientist, Hershey is perhaps best known for the 1952 "blender experiment" that he and Martha Chase conducted to demonstrate that DNA, not protein, was the genetic material of life. This discovery stimulated further research into DNA, including the discovery by **James Watson** and **Francis Crick** of the double-helix structure of DNA the following year. Hershey's work with bacteriophages, the viruses that prey on **bacteria**, was often carried out in loose collaboration with other scientists working with bacteriophages. Hershey shared the Nobel Prize in Physiology or Medicine in 1969 with Max Delbrück and Salvador Edward Luria. The Nobel Committee praised the three scientists for their contributions to **molecular biology**. Their basic research into viruses also helped others develop vaccines against viral diseases such as polio.

Hershey was born in Owosso, Michigan, to Robert Day Hershey and Alma Wilbur Hershey. Hershey's father worked for an auto manufacturer. Alfred attended public schools in Owosso and nearby Lansing. He received his B.S. in bacteriology from Michigan State College (now Michigan State University) in 1930 and his Ph.D. in chemistry from the same school in 1934. Hershey's interest in bacteriology and the **biochemistry** of life was already evident when he was a graduate student. His doctoral dissertation was on the chemistry of *Brucella,* the bacteria responsible for **brucellosis**, also known as undulant fever. After receiving his Ph.D., Hershey took a position as a research assistant in the Department of Bacteriology at the Washington University School of Medicine in St. Louis. There, he worked with Jacques Jacob

Bronfenbrenner, one of the pioneers in **bacteriophage** research in the United States. During the sixteen years he spent teaching and conducting research at Washington University, from 1934 to 1950, Hershey was promoted to instructor (1936), assistant professor (1938), and associate professor (1942).

Bacteriophages—known simply as phages—had been discovered in 1915, only nineteen years before Hershey began his career. Phages are viruses that reproduce by preying on bacteria, first attacking and then dissolving them. For scientists who study bacteria, phages are a source of irritation because they can destroy bacterial cultures. But other scientists are fascinated by this tiny organism. Perhaps the smallest living thing, phages consist of little more than the protein and DNA (the molecule of heredity) found in a cellular **nucleus**.

By studying viral replication, scientists hoped to learn more about the viral diseases that attack humans, like **mumps**, the common **cold**, German **measles**, and polio. But the study of bacteriophages also promised findings with implications that reached far beyond disease cures into the realm of understanding life itself. If Hershey and other researchers could determine how phages replicated, they stood to learn how higher organisms—including humans—passed genetic information from generation to generation.

Hershey's study of phages soon yielded several discoveries that furthered an understanding of genetic inheritance and change. In 1945, he showed that phages were capable of spontaneous mutation. Faced with a bacterial **culture** known to be resistant to phage attack, most, but not all, phages would die. By mutating, some phages survived to attack the bacteria and replicate. This finding was significant because it showed that **mutations** did not occur gradually, as one school of scientific thought believed, but immediately and spontaneously in viruses. It also helped explain why a viral attack is so difficult to prevent. In 1946, Hershey made another discovery that changed what scientists thought about viruses. He showed that if different strains of phages infected the same bacterial cell, they could combine or exchange genetic material. This is similar to what occurs when higher forms of life sexually reproduce, of course. But it was the first time viruses were shown to combine genetic material. Hershey called this phenomenon genetic **recombination**.

Hershey was not the only scientist who saw the potential in working with bacteriophages. Two other influential scientists were also pursuing the same line of investigation. Max Delbrück, a physicist, had been studying phages in the United States since he fled Nazi Germany in 1937. Studying genetic recombination independently of Hershey, he reached the same results that Hershey did in the same year. Similarly, Salvador Edward Luria, a biologist and physician who immigrated to the United States from Italy in 1940, had independently confirmed Hershey's work on spontaneous mutation in 1945. Although the three men never worked side by side in the same laboratory, they were collaborators nonetheless. Through conversation and correspondence, they shared results and encouraged each other in their phage research. Indeed, these three scientists formed the core of the self-declared "phage group," a loose-knit clique of scientists who encouraged research on particular strains of bacteriophage. By avoiding competition and duplication, the group hoped to advance phage research that much faster.

In 1950, Hershey accepted a position as a staff scientist in the department of genetics (now the Genetics Research Unit) of the Carnegie Institute at Cold Spring Harbor, New York. It was at Cold Spring Harbor that Hershey conducted his most influential experiment. Hershey wished to prove conclusively that the genetic material in phages was DNA. Analysis with an **electron microscope** had showed that phages consist only of DNA surrounded by a protein shell. Other scientists' experiments had revealed that during replication some part of the parental phages was being transferred to their offspring. The task before Hershey was to show that it was the phage DNA that was passed on to succeeding generations and that gave the signal for replication and growth.

With Martha Chase, Hershey found a way to determine what role each of the phage components played in replication. In experiments done in 1951 and 1952, Hershey used radioactive phosphorus to tag the DNA and radioactive sulfur to tag the protein. (The DNA contains no sulfur and the protein contains no phosphorus.) Hershey and Chase then allowed the marked phage particles to infect a bacterial culture and to begin the process of replication. This process was interrupted when the scientists spun the culture at a high speed in a blender.

In this manner, Hershey and Chase learned that the shearing action of the blender separated the phage protein from the bacterial cells. Apparently while the phage DNA entered the bacterium and forced it to start replicating phage particles, the phage protein remained outside, attached to the cell wall. The researchers surmised that the phage particle attached itself to the outside of a bacterium by its protein "tail" and literally injected its nucleic acid into the cell. DNA, and not protein, was responsible for communicating the genetic information needed to produce the next generation of phage.

In 1953, a year after Hershey's blender experiment, the structure of DNA was determined in Cambridge, England, by James Dewey Watson and Francis Harry Compton Crick. Watson, who was only twenty-five years old when the structure was announced, had worked with Luria at the University of Indiana. For their discovery of DNA's double-helix structure, Watson and Crick received the Nobel Prize in 1962.

Hershey, Delbrück, and Luria also received a Nobel Prize for their contributions to molecular biology, but not until 1969. This seeming delay in recognition for their accomplishments prompted the *New York Times* to ask in an October 20, 1969, editorial: "Delbrück, Hershey and Luria richly deserve their awards, but why did they have to wait so long for this recognition? Every person associated with molecular biology knows that these are the grand pioneers of the field, the giants on whom others—some of whom received the Nobel Prize years ago—depended for their own great achievements." Yet other scientists observed that the blender experiment merely offered experimental proof of a theoretical belief that was already widely held. After the blender experiment, Hershey continued investigating the structure of phage DNA. Although human DNA winds double-stranded like a spiral staircase, Hershey found that some

phage DNA is single-stranded and some is circular. In 1962, Hershey was named director of the Genetics Research Unit at Cold Spring Harbor. He retired in 1974.

Hershey was "known to his colleagues as a quiet man who avoids crowds and noise and most hectic social activities," according to the report of the 1969 Nobel Prize in the 17 October 1969 *New York Times*. His hobbies were woodworking, reading, gardening, and sailing. He married Harriet Davidson, a former research assistant, in 1945. She later became an editor of the *Cold Spring Harbor Symposia on Quantitative Biology.* Hershey and his wife had one son. Hershey died at his home in Syosset, New York, at age 89.

See also Bacteriophage and bacteriophage typing; Molecular biology and molecular genetics; Viral genetics

HETEROTROPHIC BACTERIA

Heterotrophic cells must ingest biomass to obtain their energy and nutrition. In direct contrast, autotrophs are capable of assimilating diffuse, inorganic energy and materials, and using these to synthesize biochemicals. Green plants, for example, use sunlight and simple inorganic molecules to photosynthesize organic matter. All heterotrophs have an absolute dependence on the biological products of autotrophs for their sustenance—they have no other source of nourishment.

All animals are heterotrophs, as are most **microorganisms** (the major exceptions being microscopic algae and blue-green **bacteria**). Heterotrophs can be classified according to the sorts of biomass that they eat. Animals that eat living plants are known as herbivores, while those that eat other animals are known as carnivores. Many animals eat both plants and animals, and these are known as omnivores. Animal **parasites** are a special type of carnivore that are usually much smaller than their prey, and do not usually kill the animals that they feed upon.

Heterotrophic microorganisms mostly feed upon dead plants and animals, and are known as decomposers. Some animals also specialize on feeding on dead organic matter, and are known as scavengers or detritivores. Even a few vascular plants are heterotrophic, parasitizing the roots of other plants and thereby obtaining their own nourishment. These plants, which often lack **chlorophyll**, are known as saprophytes.

Heterotrophic bacteria, therefore, are largely responsible for the process of organic matter decomposition. Many pathogenic (disease-causing) bacteria are heterotrophs. However, many species of heterotrophic bacteria are also abundant in the environment and are considered normal flora for human skin. The recycling of minerals in aquatic ecosystems, especially in estuaries, is also made possible by heterotrophic bacteria. Although monitored by health officials, the presence of heterotrophic bacteria in public water supplies is seldom considered a **public health** threat.

See also Autotrophic bacteria

Technician working with bacterial cultures.

HETEROTROPHIC PLATE COUNT • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

HIGH EFFICIENCY PARTICULATE AIR (HEPA) FILTER • *see* FUME HOOD

HISTAMINE

Histamine is a hormone that is chemically similar to the hormones serotonine, epinephrine, and norepinephrine. A hormone is generally defined as a chemical produced by a certain cell or tissue that causes a specific biological change or activity to occur in another cell or tissue located elsewhere in the body. Specifically, histamine plays a role in localized immune responses and in allergic reactions.

A select population of cells located in the brain manufactures histamine. After being made, the hormone is stored in a number of cells (e.g., mast cells, basophils, enterochromaffin cells).

Normally, there is a low level of histamine circulating in the body. However, the release of histamine can be triggered by an event such as a mosquito bite. Histamine causes the inconvenient redness, swelling and itching associated with the bite. For those with severe **allergies**, the sudden and more generalized release of histamine can be fatal (e.g., anaphylactic shock).

Mast cell histamine has an important role in the reaction of the **immune system** to the presence of a compound to which the body has developed an allergy. When released from mast cells in a reaction to a material to which the immune system is allergic, the hormone causes blood vessels to increase in diameter (e.g., vasodilation) and to become more permeable to the passage of fluid across the vessel wall. These effects are apparent as a runny nose, sneezing, and watery eyes. Other symptoms can include itching, burning and swelling in the skin, headaches, plugged sinuses, stomach cramps, and diarrhea. Histamine can also be released into the lungs, where it

causes the air passages to become constricted rather than dilated. This response occurs in an attempt to keep the offending allergenic particles from being inhaled. Unfortunately, this also makes breathing difficult. An example of such an effect of histamine occurs in asthma.

Histamine has also been shown to function as a neurotransmitter (a chemical that facilitates the transmission of impulses from one neural cell to an adjacent neural cell).

In cases of an extreme allergic reaction, adrenaline is administered to eliminate histamine from the body. For minor allergic reactions, symptoms can sometimes be lessened by the use of antihistamines that block the binding of histamine to a receptor molecule.

See also Immune system

HISTOCOMPATIBILITY

Histocompatibility refers to the means by which a eukaryotic cell can be identified. The phenomenon is the result of the presence of proteins on the surface of cells. These proteins are referred to as histocompatibility molecules. The histocompatibility molecules on the cells of one individual of a species are unique. Thus, if the cell is transplanted into another person, the cell will be recognized by the **immune system** as being foreign. The histocompatibility molecules act as an antigen in the recipient, and so can also be called a histocompatibility antigen or transplantation antigen. This is the basis of the rejection of transplanted material.

Identical twins have the same histocompatibility molecules on their cells. Thus, tissue can be successfully transplanted from one individual to the other, because the tissue will essentially not be foreign. However, for unrelated individuals, cells will have their own signature chemistry with respect to the histocompatibility molecules. Tissue from one individual will be recognized as foreign in another individual.

The suite of histocompatibility molecules present on the surface of a cell is also referred to as the histocompatibility complex. There are two classes of these molecules. The first class is called class I molecules. These molecules are made up of a portion that is embedded in the cell membrane and a portion that protrudes out from the membrane's outer surface. The protruding portion is composed of both protein and sugar (carbohydrate). Some of the human leukocyte antigens are examples of class I molecules.

The class I molecules function to chemically tag a cell so that the cell will be recognized and categorized by the T lymphocyte cells of the immune system. The T cell will recognize a region of the histocompatibility complex as "self." Because of this recognition, there will not be an immune response initiated against the cell. But, in another host, where the same region is chemically different from class I groups on the host cells, the introduced cells would be recognized as foreign by the T lymphocytes.

Another class of histocompatibility molecules called class II are anchored into the cell membrane by have two segments of the molecule. At the outer surface of the cell the molecule contains an antigen that has been acquired from the surrounding environment when particles were taken in and degraded by host processes. This is called antigen presentation.

Class II molecules are on the surface of macrophages and B-lymphocytes. These immune cells function to process cells and present the antigens from these cells to T lymphocytes. This is done to increase the repertoire of antibodies that an organism possesses. Antigen presentation of histocompatibility molecules "primes" the immune system. When an invading organism is detected, the immune response can occur much more swiftly than if no exposure to the antigen had ever occurred.

The role of the histocompatibility complexes in immune recognition of "self " and "non-self" is the reason why transplants are typically accompanied by the administration of drugs that dampen down the immune response of the host. Only by nullifying the host recognition of the class I and class II histocompatibility complexes can the transplant be maintained.

The genes that encode the histocompatibility determinants are clustered together on the chromosome. These clusters are referred to as the major and minor histocompatibility complexes. The major compatibility genes are clustered together on one chromosome. The minor compatibility genes are located in several clusters throughout the genome.

Studies on mice, which also possess the histocompatibility complexes, have demonstrated that these complexes not only play a role in transplant rejection, but also function in the immune response to a variety of diseases. Mice that are genetically different for a given histocompatibility complex will respond differently to the same antigen. If a "non-self" histocompatibility complex is poorly recognized by the host immune system, then an inadequate immune response will ensue. The result can be the establishment of an infection.

See also Antibody and antigen; Immune system; Immunodeficiency diseases; Major histocompatibility complex (MHC)

HISTORY OF IMMUNOLOGY

In Western society, it was not until the late eighteenth century that a rational approach to the origin of disease developed. Prior to the discovery that disease was the result of pathogenic organisms, it was commonly accepted that disease was a punishment from God (or the Gods), or even a witches curse. Eastern cultures perceived disease as an imbalance in the energy channels within the body. Later, the great plagues of Europe were assumed the result of virulent or noxious vapors. Nevertheless, there were intimations as early as 430 B.C. that if one survived a disease, the person thereafter became "immune" to any subsequent exposures. However, this was never recognized as evidence of some type of internal defense system until the later part of the seventeenth century.

Although most historical accounts credit **Edward Jenner** for the development of the first **immunization** process, a previous similar procedure had become established in China by 1700. The technique was called variolation. This was derived

from the name of the infective agent—the **variola virus**. The basic principal of variolation was to deliberately cause a mild infection with unmodified pathogen. The risk of death from variolation was around two to three percent. Although still a risk, variolation was a considerable improvement on the death rate for uncontrolled infection. **Immunity** to **smallpox** was conferred by inserting the dried exudate of smallpox pustules into the nose. This technique for the transfer of smallpox, as a form of limited infection, traveled to the west from China along the traditional trade routes to Constantinople where it spread throughout Europe. Hearing of this practice, the Royal family of England had their children inoculated against the disease in 1721, but the practice aroused severe opposition as physicians felt it was far too risky.

In 1798, Edward Jenner, noticed that milkmaids were protected from smallpox if they had been first infected with **cowpox**. It was not his intention to make medical history, as his interests were mostly scholarly and involved the transfer of infections from one species to another, especially from animals to humans. However, Jenner's work led him to the conclusion, that inoculation with cowpox (a bovine analogue of smallpox) could confer immunity to smallpox. Thus, the concept of **vaccination** was initiated. (Incidentally, the Latin word for cow is *vacca*). Jenner's ideas first made him a medical as well as a social pariah, as they were in opposition to both the church and popular beliefs. Because his method was much safer then variolation, however, the use of vaccinations gradually became widely accepted and most European countries had some form of compulsory program within fifty years of Jenner's discovery.

The idea that a pathogenic organism caused disease was not fully realized until certain technological advances had occurred. Initially, **Antoni van Leeuwenhoek**'s development of the **microscope** and the subsequent realization that entities existed that were not visible to the human eye, allowed the concept of germs to be appreciated. That these organisms were the causative agent of disease was not recognized until **Louis Pasteur** developed his **germ theory of disease**. His original interests were in **fermentation** in wine and beer, and he was the first to isolate the organisms that caused the fermentation process. Pasteur's work eventually led him to the development of **pasteurization** (heating) as a means of halting fermentation. While working with silk worms and **anthrax**, he was able to demonstrate that the same method for transferring the fermentation process also worked in transmitting disease from infected animals to unaffected animals. Finally, in 1878, Pasteur accidentally used an attenuated (weakened) chicken cholera **culture** and realized, when he repeated the experiment using a fresh culture, that the weakened form protected the chickens from the virulent form of the disease. Pasteur went on to develop an attenuated **vaccine** against **rabies** and swine erysipelas.

Pasteur was not the only proponent of the germ theory of disease. His chief competitor was **Robert Koch**. Koch was the first to isolate the anthrax microbe and, unaware of Pasteur's work, he was able to show that it caused the disease. Then in 1882, Koch was able to demonstrate that the germ theory of disease applied to human ailments as well as animals, when he isolated the microbe that caused **tuberculosis**. His "Koch's postulates" are still used to identify infective organisms.

Much of the basis for modern medicine, as well as the field of **immunology**, can be traced back to these two scientists, but the two major questions still to be answered were how did infection cause the degradation of tissue, and how did vaccines work? The first question was addressed in 1881 by **Emile Roux** and Alexander Yersin when they isolated a soluble toxin from **diphtheria** cultures. Later, **Emil von Behring** and **Shibasaburo Kitasato** were able to demonstrate passive immunity when they took serum from animals infected with diphtheria and injected into healthy animals. These same animals were found to be resistant to the disease. Eventually these serum factors were recognized in 1930 as antibodies. However, thirty years before antibodies were finally isolated and identified, **Paul Ehrlich** and others, recognized that a specific antigen elicited the production of a specific **antibody**. Ehrlich hypothesized that these antibodies were specialized molecular structures with specific receptor sites that fit each pathogen like a lock and key. Thus, the first realization that the body had a specific defense system was introduced. In addition, sometime later, he realized that this powerful effector mechanism, used in host defense would, if turned against the host, cause severe tissue damage. Ehrlich termed this *horror autotoxicus*. Although extremely valuable, his work still left a large gap in understanding how the **immune system** fights a pathogenic challenge. The idea that specific cells could be directly involved with defending the body was first suggested in 1884 by **Élie Metchnikoff**. His field was zoology and he studied **phagocytosis** in single cell organisms. Metchnikoff postulated that vertebrates could operate in a similar manner to remove pathogens. However, it was not until the 1940s that his theories were accepted and the cell mediated, as opposed to the humoral, immune response was recognized.

The clarification of the immune response and the science of immunology did not progress in a systematic or chronological order. Nonetheless, once scientists had a basic understanding of the cellular and humoral branches of the immune system, what remained was the identification of the various components of this intricate system, and the mechanisms of their interactions. This could not have been accomplished without the concomitant development of **molecular biology** and genetics.

Milestones in the history of immunology include:

1798 Edward Jenner initiates smallpox vaccination.

1877 Paul Erlich recognizes mast cells.

1879 Louis Pasteur develops an attenuated chicken cholera vaccine.

1883 Elie Metchnikoff develops cellular theory of vaccination.

1885 Louis Pasteur develops rabies vaccine.

1891 Robert Koch explored delayed type hypersensitivity.

1900 Paul Erlich theorizes specific antibody formation.

1906 Clemens von Pirquet coined the word allergy.

1938 John Marrack formulates antigen-antibody binding hypothesis.

1942 Jules Freund and Katherine McDermott research

adjuvants.

1949 Macfarlane Burnet & Frank Fenner formulate immunological tolerance hypothesis.

1959 Niels Jerne, David Talmage, Macfarlane Burnet develop clonal **selection** theory.

1957 Alick Isaacs & Jean Lindemann discover interferon (cytokine).

1962 Rodney Porter and team discovery the structure of antibodies.

1962 Jaques Miller and team discover thymus involvement in cellular immunity.

1962 Noel Warner and team distinguish between cellular and humoral immune responses.

1968 Anthony Davis and team discover T cell and B cell cooperation in immune response.

1974 Rolf Zinkernagel and Peter Doherty explore **major histocompatibility complex** restriction.

1985 Susumu Tonegawa, Leroy Hood, and team identify immunoglobulin genes.

1987 Leroy Hood and team identify genes for the T cell receptor.

1985 Scientists begin the rapid identification of genes for immune cells that continues to the present.

See also Antibody and antigen; B cells or B lymphocytes; Germ theory of disease; History of the development of antibiotics; History of public health; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Infection and resistance; T cells or T-lymphocytes

History of Microbiology

Microbiology was born in 1674 when **Antoni van Leeuwenhoek** (1632–1723), a Dutch drapery merchant, peered at a drop of lake water through a carefully ground glass lens. Through this he beheld the first glimpse of the microbial world. Perhaps more than any other science, the development of microbiology depended on the invention and improvement of a tool, the **microscope**. Since **bacteria** cannot be seen individually with the unaided eye, their existence as individuals can only be known through microscopic observations. Indeed, it is interesting to speculate on how microbiology might have developed if the limits of resolution of the microscope were poorer.

The practical and scientific aspects of microbiology have been closely woven from the very beginning. Perhaps it is for this reason that microbiology as a field of study did not really develop until the twentieth century. Nineteenth century "microbiologists" were chemists and physicians and a few were botanists. At that stage, the science of microbes was developing to solve very practical problems in two clear scientific fields, the science of **fermentation** and in medicine.

Although medicine and fermentation presented the practical problems that stimulated the development of microbiology, the first studies that put the subject on a scientific basis arose from a problem of pure science. This was the controversy over spontaneous generation. Although the crude ideas of spontaneous generation (e.g., maggots from meat) were dispelled by Francesco Redi (1626?–1698?) in the seventeenth century, more subtle ideas such as that **protozoa** and bacteria can arise from vegetable and animal infusions, were still accepted in the nineteenth century. The controversy also involved fermentations, since it was considered that the **yeast** fermentation was of spontaneous origin.

Many workers became involved in the study of fermentation and spontaneous generation, but **Louis Pasteur** (1822–1895) stands out as a giant. He came into biology from the field of chemistry and was apparently able to remove all the philosophical hurdles that blocked the thinking of others. Within a period of four years after he began his studies, he had clarified the problems of spontaneous generation so well that the controversy died a natural death.

Pasteur was also able to go easily from fermentation into the field of medical microbiology, which occupied the later part of his life. His contributions in that field were numerous, and his work in fields such as microbial attenuation and **vaccination** has been the basis of many modern medical practices. It should be emphasized that the development of **sterilization** methods by researchers such as Pasteur and John Tyndall (1820–1893), so necessary to the solution of the spontaneous generation controversy, were essential to put the science of microbiology on a firm foundation. The workers did not set out to develop these methods, but they evolved as a bonus that was received for solving the spontaneous generation question.

Other important developments were in medicine. The microbiological aspects of medicine arose out of considerations of the nature of contagious disease. Although the phenomenon of contagion, especially with respect to diseases such as **smallpox**, was recognized far back in antiquity, its nature and relationship to **microorganisms** was not understood. It was probably the introduction of **syphilis** into Europe, which served to crystallize thinking as here was a disease that could only be transmitted by contact and helped to formulate the question, what is being transmitted? Gerolamo Fracastoro (1478–1553) gave syphilis its name in the sixteenth century and came close to devising a **germ theory of disease**, an idea that later attracted a number of workers all the way down to the nineteenth century. By the late 1830s, Schwann and Cagniard-Latour had shown that alcoholic fermentation and putrefaction were due to living, organized beings. If one accepted the fact that the decomposition of organic materials was due to living organisms, it was only a step further to reason that disease, which in many ways appears as the decomposition of body tissues, was due to living agents. Jacob Henle, in 1840, further commented on this similarity and with the newfound knowledge on the nature of fermentation, he proceeded to draw rather clear conclusions also saying that experimental proof would be required to clinch this hypothesis. That evidence came later from **Robert Koch** provided, in 1867, the final evidence proving the germ theory. He established the etiologic role of bacteria in **anthrax** and as a result proposed a set of rules to be followed in the establishment of etiology. The key to Koch's observation was the isolation of

Robert Koch in his laboratory in the late nineteenth century.

the organism in pure **culture**. While limiting dilutions could have been used (as described previously by **Joseph Lister**, 1827–1912), Koch promoted the use of solid media, giving rise to separate colonies and the use of stains. In 1882, Koch identified the tubercle bacillus and so formalized the criteria of Henle for distinguishing causative pathogenic microbes. This set of criteria is known as **Koch's postulates**.

One of the most important applied developments in microbiology was in understanding the nature of specific acquired **immunity** to disease. That such immunity was possible was known for a long time, and the knowledge finally crystallized with the prophylactic treatment for smallpox introduced by **Edward Jenner** (1749–1823). Using **cowpox**, Jenner introduced the first vaccination procedures in 1796. This occurred long before the germ theory of disease had been established. Later workers developed additional methods of increasing the immunity of an individual to disease, but the most dramatic triumph was the discovery of the **diphtheria** and **tetanus** antitoxins by von Behring and Kitisato in the 1890s. This work later developed into a practical tool by **Paul Ehrlich** (1854–1915) and it was now possible to cure a person suffering from these diseases by injecting some antitoxic serum prepared by earlier **immunization** of a horse or other large animal. This led for the first time to rational cures for infectious diseases, and was responsible for Ehrlich's later conception of **chemotherapy**. The **antibiotics** era, which followed the groundbreaking work of **Alexander Fleming** (1881–1955) with **penicillin**, was another important step in the understanding of microbiology.

Most of the most recent work in the development of microbiology has been in the field of **microbial genetics** and how it evolved into a separate discipline known as **molecular biology**. This work really began in the 1940s, when **Oswald Avery, Colin MacLeod** and **Maclyn McCarty** demonstrated that the transforming principle in bacteria, previously observed by Frederick Griffiths in 1928, was **DNA**. **Joshua Lederberg** and **Edward Tatum** demonstrated that DNA could be transferred from one bacterium to another in 1944. With the determination of the structure of DNA in 1953, a new and practical aspect of microbiology suddenly became realised, and the foundations of genetic engineering were laid. It is perhaps important to realize that if it were not for bacteria and their characteristics, genetic engineering would not be possible. The concept of DNA transfer was essentially born in the 1940s. Later on, in the late 1960s bacterial **restriction enzymes** were discovered and the possibilities of splicing and rearranging DNA emerged. The advances in molecular biology following these major breakthroughs have been immense but it is important to realize that the field of microbiology lies at their root.

See also Antibiotics; Fermentation; Microscope and microscopy; Vaccine

History of Public Health

Infections caused by **microorganisms** are often spread more easily in an unsanitary environment. Even today, for example, ***Escherichia coli*** infections of food and between people are still commonly caused by poor hygienic practices, such as the failure to properly wash hands after toileting.

Because of the association between microbial infection and sanitary conditions, many **public health** initiatives and regulations have been instituted around the world. Before the recognition that microbes caused infections, and even before the realization that microbes existed, public health was a foreign concept. The history of public health parallels advances in the understanding of microorganisms and disease.

Prior to the fourteenth century, public health was nonexistent. The sanitary environment in urban centers was appalling by today's standards. However, at that time there was no knowledge that, for example, the flow of raw sewage alongside streets was connected to illness. The occurrence of **bubonic plague** in Europe in 1348 began to change this view. Between 1348 and 1350, the infection caused by *Yersinia pestis* killed almost two-thirds of the population in the major urban centers of Europe. In the aftermath of this devastation came an increased awareness of the influence of health conditions and disease. In the 1350s, Italian government initiatives sought to improve sanitary and living conditions. These initiatives occurred even though the existence of microorganisms was not

The scourge of epidemics.

yet known. By the sixteenth century, the idea of a microbial cause of disease (e.g., "contagion") was being debated.

In the United States, the organized public health initiative that is today's Public Health Service began in the late eighteenth century. Legislation passed by the United States Congress in 1798 provided care and relief for afflicted mariners. Hospitals to serve seamen were established on the eastern seaboard and later in cities on the Great Lakes, Gulf coast and Pacific coast. In 1870, the control of these hospitals became the responsibility of the newly formed Marine Hospital Service, headquartered in Washington, DC.

Because mariners were often the carriers of infectious diseases acquired in other countries, the Marine Hospital Service soon became concerned with infectious diseases. In 1887, a small bacteriology laboratory (the Hygienic Laboratory) was created at a marine hospital on Staten Island in the New York harbor. The laboratory was later relocated to Washington, DC, where it became the National Institutes of Health.

The increasing responsibility for infectious disease treatment and research prompted a name change of the organization to the Public Health and Marine Service (1902) and the Public Health Service (1912). In the twentieth century, the services' role in controlling infectious microbial diseases and funding infectious disease research expanded. For example, among the agencies that comprise the Public Health Service are the **Centers for Disease Control**, the National Institutes of Health, and the Food and Drug Administration. Approximately 60,000 people are employees of the Public Health Service, whose annual budget now exceeds 15 billion dollars.

As the public health infrastructure was growing in the nineteenth century, public health nursing was also growing in the United States. In 1898, Los Angeles became the first city to employ a nurse to care for sick people in their homes. Thereafter, more governments recognized that caring for the populace in the homes and workplaces had a positive effect on the society as a whole, in terms of reducing the spread of infectious diseases.

During the nineteenth century, microbiologists such as **Louis Pasteur** and **Robert Koch** demonstrated the involvement of **bacteria** in disease. The importance of maintaining a hygienic atmosphere in hospitals was recognized. Indeed, **Joseph Lister**'s implementation of the use of a disinfectant spray and fresh changes of operating smocks for surgeons greatly decreased the mortality rate associated with operations. Because of these public health efforts, operations moved from a position of last resort to a procedure that could improve the health and alleviate suffering in people.

The increasing disparity between the developed and underdeveloped countries of the world spawned major international public health efforts in the twenty-first century. The most prominent example is the **World Health Organization** (**WHO**), created in 1948. Other agencies are noteworthy as well, such as the United Nations International Children's Emergency Fund (UNICEF), which was created in 1946 to aid children victimized by World War II.

At the time of the creation of UNICEF and the WHO, the average life expectancy in the world was 46 years. As of the late 1990s, this average life expectancy had risen to 65 years of age. Much of this increase is attributable to organized public health initiatives such as **vaccination**, medical care and hydration programs.

The recognition of the involvement of microorganisms and disease has continued to the present day. Since the 1970s, a series of microbiologically related diseases (i.e., Acquired **Immunodeficiency** Syndrome) has prompted renewed emphasis on public health. Other diseases that were thought to be due to a genetic or physiological abnormality (e.g., stomach ulcers, heart disease) have been demonstrated to be at least partially due to chronic bacterial infections. Lessening the chances of exposure to such infectious agents has become another public health concern.

Another watershed in the history of public health has occurred in the latter decades of the twenty-first century. Then, the importance of the adherent bacterial populations known as biofilms to the establishment and maintenance of long-lasting infections that are extremely resistant to treatments became known. **Biofilms** are important in environments ranging from the operating room to the drinking water treatment plant. This importance has focused public health efforts on the prevention of biofilm build-up and on the creation of strategies that eliminate the biofilm bacteria.

See also AIDS; Bacteria and bacterial infection; Epidemics and pandemics; Infection control; Public health, current issues

HISTORY OF THE DEVELOPMENT OF ANTIBIOTICS

The great modern advances in **chemotherapy** have come from the chance discovery that many **microorganisms** synthesize and excrete compounds that are selectively toxic to other microorganisms. These compounds are called **antibiotics** and have revolutionized medicine. The period since World War II has seen the establishment and extremely rapid growth of a major industry, using microorganisms for the synthesis of, amongst other compounds, chemotherapeutic agents. The development of this industry has had a dramatic and far-reaching impact. Nearly all bacterial infectious diseases that were, prior to the antibiotic era, major causes of human death have been brought under control by the use of chemotherapeutic drugs, including antibiotics. In the United States, bacterial infection is now a less frequent cause of death than suicide or traffic accidents.

The first chemotherapeutically effective antibiotic was discovered in 1929 by **Alexander Fleming** (1881–1955), a British bacteriologist, who had long been interested in the treatment of wound infections. On returning from a vacation in the countryside, he noticed among a pile of petri dishes on his bench one that had been streaked with a **culture** of *Saphyloccocus aureus* which was also contaminated by a single **colony** of **mold**. As Fleming observed the plate, he noted that the colonies immediately surrounding the mold were transparent and appeared to by undergoing lysis. He reasoned that the mould was excreting into the medium a chemical that caused the surrounding colonies to lyse. Sensing the possible chemotherapeutic significance of his observation, Fleming isolated the mold, which proved to be a species of *Penicillium,* and established that culture filtrates contained an antibacterial substance, which he called **penicillin**.

Although it has often been suggested that many bacteriologists must have observed petri dishes that were similarly contaminated and therefore similar in appearance to Fleming's dish, such speculation is undoubtedly false. As subsequent experiments have shown, a highly unusual series of events must have occurred in order to produce the results seen on Fleming's plate: **contamination** must have occurred at the time the plate was streaked with **bacteria** (prior growth of either would have prevented growth of the other in the immediate vicinity); the inoculated petri dish must not have been incubated (if it had been the bacterium would have outgrown the mold); the room temperature of the laboratory must have been below 68°F [20° C] (a temperature that probably did occur during a brief cold storm in London in the summer of 1928).

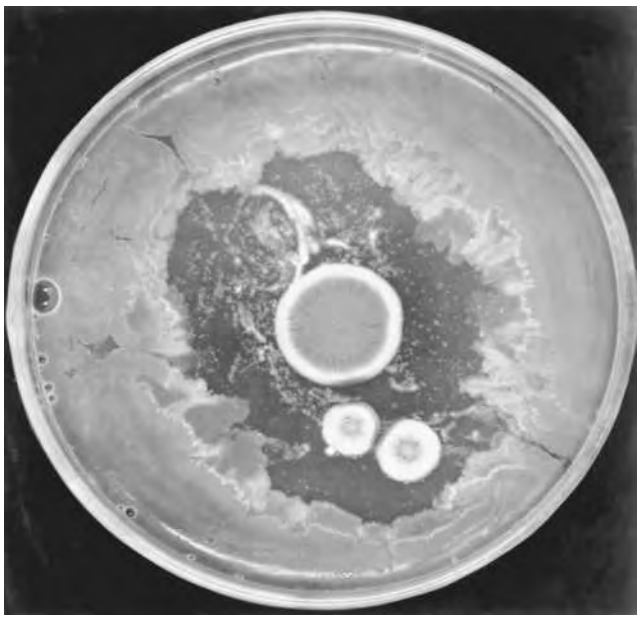
Penicillin proved to be chemically unstable and Fleming was unable to purify it. Working with impure preparations, he demonstrated its remarkable effectiveness in inhibiting the growth of many Gram-positive bacteria, and he even used it with success for the local treatment of human **eye infections**. In the meantime, the chemotherapeutic effectiveness of other, non-antibiotic compounds such as sulfonamides had been discovered, and Fleming, discouraged by the difficulties in purifying penicillin, abandoned further work on the problem.

Ten years later a group of British scientists headed by **H.W. Florey** (1898–1968) and **E. Chain** (1906–1979) resumed the study of penicillin. Clinical trials with partly purified material were dramatically successful. By this time, however, Britain was at war; and the industrial development of penicillin was undertaken in the United States, where an intensive program of research and development was begun in many laboratories. Within three years, penicillin was being produced on an industrial scale. Today it remains one of the most effective chemotherapeutic agents for the treatment of many bacterial infections.

Rather than being a single substance, penicillin turned out to be a class of compounds. The various penicillins vary with respect to the chemical composition of their side chain. The penicillin that was first isolated in Peoria, Illinois, designated penicillin G, carried a benzyl side chain. The penicillin isolated soon after in England, designated penicillin F, carried an isopentanyl side chain. By varying the composition of the fungal growth media, a variety of penicillins collectively termed biosynthetic penicillins, have been synthesized. Penicillin G proved the most successful and later it became possible to remove the side chain and replace it by a variety of chemical substituents, thereby producing semisynthetic penicillins. For example, penicillin V is resistant to acid and therefore can be administered orally because it is not inactivated in the stomach; ampicillin is also acid resistant and also effective against enteric bacteria; oxacillin is resistant to the action of B-lactamase, the enzyme produced by certain "penicillin-resistant" strains of bacteria.

The remarkable chemotherapeutic efficacy of penicillin for certain bacterial infections, primarily those caused by Gram-positive bacteria, prompted intensive research into new antibiotics. In the 1940s, a second clinically important antibiotic, streptomycin, effective against both Gram-negative bacteria and *Mycobacterium tuberculosis,* was discovered by A. Schatz and **S. Waksman**. This was the first example of a broad-spectrum antibiotic. Other antibiotics with even broader spectra of activity, such as the tetracyclines, were subsequently discovered. The search for new antibiotics remains an empirical enterprise. So far, they have proved very effective as antibacterial agents, although some bacteria do acquire resistance to antibiotics, so there is a continuous search for new and effective antibacterial agents. Antibiotics have proved less effective in the treatment of fungal infections. Antifungal antibiotics, such as nystatin and amphoterecin B are considerably less successful therapeutically than their bacterial counterparts, at least in part because their toxicity is far less selective. There are no known antiviral antibiotics.

Since 1945, thousands of different antibiotics produced by **fungi**, actinomycetes or unicellular bacteria have been isolated and characterized. A small fraction of these are of therapeutic value. Their nomenclature is complicated as one antibiotic may be sold under several different names. For example in the United States the compound, which in Europe



Photograph of the original culture plate of the fungus *Penicillum notatum* made by the Scottish bacteriologist Alexander Fleming in 1928.

has the generic name rifampicin, is called rifampin. Its proper chemical class name is rifamycin and it is also sold under the trade names Rifactin and Rifadin, among others.

See also Bacteria and bacterial infection; Fungicide; History of microbiology; History of public health; Streptococci and streptococcal infections; Sulfa drugs

HIV • *see* HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HOBBY, GLADYS LOUNSBURY (1910-1993)

American microbiologist

Gladys Lounsbury Hobby was one of the few women who were part of the extensive network that brought **penicillin** from the laboratory to the clinic. Discovered by Sir **Alexander Fleming** in 1928, penicillin was one of the first **antibiotics**. In her book, *Penicillin: Meeting the Challenge,* Hobby detailed the efforts in the early 1940s to discover a way to manufacture large amounts of penicillin, which would greatly aid in the treating of war wounded. In addition to her work as a microbiologist, Hobby wrote many articles and was a teacher.

Hobby was born November 19, 1910, in New York City. She received her Bachelor of Arts degree from Vassar College in 1931; she then attended Columbia University, receiving her master's degree in 1932 and her doctorate in bacteriology three years later. From 1934 to 1943, she worked on perfecting penicillin specifically for several infectious diseases as part of a research team at the Columbia Medical School, while also being professionally involved at Presbyterian Hospital in New York City. In 1944, Hobby went to work for Pfizer Pharmaceuticals in New York, where she researched streptomycin and other antibiotics, discovering how antimicrobial drugs worked. In 1959, Hobby became chief of research at the Veteran's Administration Hospital in East Orange, New Jersey, where she worked on chronic infectious diseases. Before retiring in 1977, she was assistant research clinical professor in **public health** at Cornell Medical College.

Retirement for Hobby meant continuing her work. Hobby became a freelance science writer and a consultant. It was during this time that she penned her book, *Penicillin: Meeting the Challenge,* about the drug's odyssey from the laboratory to the hands of the clinician. Hobby, having taken meticulous notes, detailed each researcher's contribution to producing a safe penicillin on a large scale basis. She also authored more than two hundred articles and was the founder and editor of the journal *Antimicrobial Agents and Chemotherapy.*

Hobby was a member of several professional organizations, including the American Association for the Advancement of Science, the American Academy of Microbiology, and the American Society of Microbiology. Hobby died suddenly of a heart attack on July 4, 1993, at her home in Pennsylvania.

See also History of the development of antibiotics

HOLDFAST • *see* CAULOBACTER

HOOKE, ROBERT (1635-1703)

English physicist

One of the preeminent scientists of the seventeenth century, Robert Hooke is perhaps best remembered for the wide variety of fields to which he contributed, including physics, astronomy, microscopy, biology, and architecture, among others. Although Hooke introduced many concepts previously unimagined or unexamined, his ability to formulate these ideas usually did not match his intuition, and the credit for many scientific breakthroughs inspired by Hooke's ideas is often given to such scientists as Isaac Newton and Christiaan Huygens, who brought the work to its fruition. Still, Hooke remains an important pioneer of science.

Born on Britain's Isle of Wight, Hooke was a sickly child. As a youth, his perpetual ill health made it impossible for him to attend classes regularly, and he was unable to enter the ministry as his father, a minister, had wished. Instead, Hooke was allowed to pursue his interest in mechanics, which he first demonstrated as a small child by constructing elaborate toys. He attended Westminster School and later Oxford, where he became the laboratory assistant to Robert Boyle. It was in Boyle' s lab that Hooke's talent for designing scientific instruments was noticed, as he constructed the improved air

pump used to establish Boyle's gas laws. In fact, it has been speculated that Hooke himself may have been the author of Boyle's law, since, customarily, any findings from research done in the lab would have been credited to the professor.

Along with some of his colleagues from Oxford and the surrounding area, Hooke helped to establish what would soon become the Royal Society, to which he was appointed Curator of Experiments. During his time as Curator he had many other successes attributed to him such as the compound **microscope**, an improved barometer, the reflecting telescope, and the universal joint.

Although Hooke was not the first to experiment using a microscope, he was the first to dedicate a major intensive volume to microscopy. His 1665 publication *Micrographia* describes the structures of insects, fossils, and plants in unprecedented detail. While studying the porous structure of cork, Hooke noted the presence of tiny rectangular holes that he called cells, a word that has been adopted as the cornerstone of microbiology. *Micrographia* also contains illustrations in Hooke's own hand that remain among the best renderings of microscopic views.

In the years following the great London fire of 1666, Hooke became a surveyor and, eventually, an architect, constructing numerous famous buildings. Because his architectural interests took much time away from his scientific work, he was ultimately forced to retire as Curator of Experiments for the Royal Society in favor of his new vocation.

See also History of microbiology; Microscope and microscopy

HTLV • *see* HUMAN T-CELL LEUKEMIA VIRUS (HTLV)

HUANG, ALICE SHIH-HOU (1939-)

Chinese-born American microbiologist

Alice Shih-hou Huang's discovery of reverse transcriptase, an enzyme that allows **viruses** to convert their genetic material into **DNA** (**deoxyribonucleic acid**)—the molecular basis of heredity—led to a major breakthrough in understanding how viruses function. Searching for clues on how to prevent viruses from replicating, Huang also isolated a rabies-like virus that produced mutant strains that interfered with viral growth.

The youngest of four children, Huang was born in Kiangsi, China, on March 22, 1939. Her father, the Right Reverend Quentin K. Y. Huang, was the second Chinese bishop ordained by the Anglican Episcopal Ministry in China. Her mother, Grace Betty Soong Huang, undertook a career of her own by entering nursing school at the age of forty-five. In 1949, when communism pervaded China, the Huangs sent their children to the United States, hoping for a more stable life and greater opportunities.

Huang was ten years old when she arrived in the United States. She studied at an Episcopalian boarding school for girls in Burlington, New Jersey, and at the National Cathedral School in Washington, D.C., and became a United States citizen her senior year in high school. While in China, Huang had seen many people suffering from illness and decided to become a physician. She attended Wellesley College in Massachusetts from 1957 to 1959, and subsequently enrolled in a special program at the Johns Hopkins University School of Medicine, where she earned her B.A. in 1961 and her M.A. in 1963. While at Johns Hopkins, she chose to pursue medicine not as a physician, but as a microbiologist. She published several papers on viruses, including the **herpes** simplex viruses, and earned her Ph.D. from Johns Hopkins in 1966. That same year Huang served as a visiting assistant professor at the National Taiwan University. In 1967, Huang worked as a postdoctoral fellow with **David Baltimore** at the Salk Institute for Biological Studies in San Diego, California. Huang and Baltimore married in 1968; they have one daughter.

Huang and Baltimore took their work to the Massachusetts Institute of Technology in 1968. At the time, scientists understood that the genetic material DNA in cells was converted into **ribonucleic acid** (**RNA**, nucleic acids associated with the control of chemical activities within cells), and then into proteins. But one of the viruses Huang studied had an enzyme that did something different—it made RNA from RNA. The work led to Baltimore's research on **tumor viruses** and his discovery of the enzyme called reverse transcriptase, which threw the usual process in reverse by converting RNA to DNA. Baltimore and American oncologist Howard Temin, who had independently discovered reverse transcriptase, were awarded the Nobel Prize in medicine in 1975 for their work on tumor viruses.

Huang became assistant professor of microbiology and **molecular genetics** at Harvard Medical School in 1971, was promoted to associate professor in 1973, and to full professor in 1979. She also served as an associate at the Boston City Hospital from 1971 to 1973 and director of the infectious diseases laboratory at the Children's Hospital in Boston from 1979 to 1989. Huang studied a rabies-like virus that produced mutant strains, which interfered with further growth of the viral infection. She sought to understand where the **mutants** originated and how they affected the viral population, knowledge she hoped could be applied to halt the spread of viral infections in humans. For this research, Huang was awarded the Eli Lilly Award in Microbiology and **Immunology** in 1977. In 1987, she was appointed trustee of the University of Massachusetts. The following year Huang became president of the American Society for Microbiology, the first Asian American to head a national scientific society in the United States. She is also a member of the American Association for the Advancement of Science, the American Society for **Biochemistry** and **Molecular Biology**, and the Academia Sinica in Taiwan. Huang remained at Harvard until 1991, when she was appointed Dean for Science at New York University.

Though Huang sees her role in administration at New York University as important and necessary, her first love remains basic research. Huang has numerous research publications to her credit, and has served on the editorial boards of *Intervirology, Journal of Virology, Reviews of Infectious Diseases, Microbial Pathogenesis,* and *Journal of Women's*

Health. She became a trustee of Johns Hopkins University in 1992, and joined the council of the Johns Hopkins-Nanjing University Center for Chinese and American Studies in 1993. In addition to her duties as university administrator, scientist, and mother, Huang is an avid reader of mystery novels, and enjoys sailing.

See also Viral genetics; Virology; Virus replication; Viruses and responses to viral infection

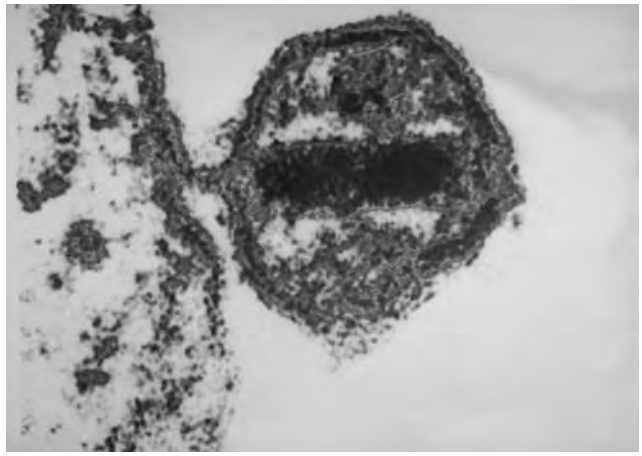
HUMAN IMMUNODEFICIENCY VIRUS (HIV)

The Human **Immunodeficiency** Virus (HIV) belongs to a class of **viruses** known as the **retroviruses.** These viruses are known as **RNA** viruses because they have RNA as their basic genetic material instead of **DNA**. The retroviruses are unable to replicate outside of living host cells, because they contain only RNA. However, they have the enzyme reverse transcriptase that can make DNA from the RNA and allow them to integrate into the host cell genome. The retroviruses are composed of three subgroups, two of which are pathogenic to humans. They are the oncarnovirus subgroup and the lentivirus (meaning, slow virus) subgroup. The Human Immunodeficiency Virus, which belongs to the lentivirus subgroup, is further divided into two types based on the diseases they produce. The HIV-1 produces the acquired immunodeficiency syndrome (**AIDS**), while the HIV-2 produces a similar disease that is at present, largely restricted to West Africa.

The genetic material of the HIV virus consists of two short strands of RNA about 9,200 nucleotides long, enclosed in an outer lipid envelope. A viral glycoprotein (gp120) is displayed on the surface of the envelope. This protein recognizes and binds to the CD4 receptor on T-helper cells. The HIV genome contains a long terminal repeat (LTR) and the gag, pol, env, and tax/rex genes. The LTR helps in the integration of the virus into the host cell DNA. The gag **gene** codes for the proteins that make up the outer core or capsid while the env gene codes for the envelope glycoprotein including the outer envelope glycoprotein (gp 120) and the transmembrane glycoprotein (gp141). The major proteins coded by the pol gene are the reverse transcriptase, protease, and the integrase. The tax/rex gene codes for certain factors that have a regulatory role.

The HIV infects cells that have the CD4 receptor molecule on their surface. In macrophages and cells lacking this molecule, an alternate receptor molecule (such as the Fc receptor, or the **complement** receptor site) may be used for entry of HIV. The immune cells such as the blood monocytes, macrophages, **T cells**, **B cells**, natural killer (NK) cells, dendritic cells, hematopoietic stem cells, etc are the primary targets of HIV infection.

After entering the body, the virus attaches itself by fusion to a cell with the appropriate CD4 receptor molecule. On gaining entry into the cell, the viral particle uncoats from its envelope and releases the RNA. The reverse transcriptase encoded by the pol gene, reverse transcribes the viral RNA into DNA, and the integrase enzyme (also coded by the pol gene) inserts the HIV proviral DNA into the genomic DNA of the host cell. The HIV provirus is replicated by the host cell and transcribed to produce new progeny RNA molecules. The infected host cells either release the new HIV virions by lysis, or the viruses can escape by surface budding. These go on to infect additional host cells.



Color enhanced scanning electron microscope image of the Human Immunodeficiency Virus (HIV) on a hemocyte.

The primary target of the HIV is the **immune system** itself, with a special affinity for CD4 (T-helper) cells. Following infection, there is a latent phase during which the viral replication continues actively, accompanied with a progressive destruction of the CD4 cells. During latency, there are enough immune cells remaining to provide an immune response and fight infections. Eventually, when a significant number of T cells are destroyed, and the rate of production of the cells cannot match the rate of destruction, there is a loss of both cell-mediated and humoral **immunity**. This failure of the immune system leads to the appearance of clinical AIDS. The patients generally die of secondary causes such as Kaposi's sarcoma (a rare form of cancer that occurs in HIV-infected individuals) or bacterial and fungal infections.

Primary HIV infection may go undetected in more than half the cases, because the symptoms produced are mild and they subside quickly. This is followed by a clinical latent period, which could last on an average 8–11 years. The latency period varies from person to person and depends on a variety of factors including the person's health status and life style. In cases of acute HIV infection, the most common symptoms are fever, swelling of the lymph glands, a red, diffuse rash all over the body, sore throat or upper respiratory infection, muscle ache, diarrhea, and headache. These symptoms subside in a couple of months. Within three months of infection, the body mounts an additional immune response to the virus, and detectable levels of antibodies are seen. Both humoral and cell-mediated immune responses play a role. There is a decline in the viral counts and the levels of CD4 T-helper cells increase. In rare cases, it may take as long as six months for the immune response to develop. Therefore, the **Centers for Disease Control** (CDC) recommends testing for HIV at six

months after the last possible exposure to the virus (through unprotected sex or sharing needles).

HIV is primarily spread as a **sexually transmitted disease**. However, one can also acquire the virus through either intravenous drug use or transfusions. The virus can be present in a variety of body fluids and secretions, but the presence of HIV in blood, and genital secretions, and to a lesser extent breast milk, is significant for the spread of HIV. In addition, HIV infection can be acquired as a congenital infection during birth or in infancy. Mothers with HIV infection can pass the virus either transplacentally at the time of delivery through the birth canal or through breast milk. The diagnosis of clinical AIDS often occurs because of the presence of rare diseases such as Kaposi's sarcoma, **pneumonia**, or other serious recurrent infections. The patient's lifestyle, and medical history could also provide clues. Laboratory diagnosis of the infection is based on **serology**, measuring the antibodies to HIV using **ELISA**. Positive results are further confirmed with another test known as a Western Blot. Together, the two tests are more than 99.9% accurate.

No vaccines are currently available to prevent infection by HIV. However, scientists and researchers the world over are working on making a **vaccine** to HIV and have some interesting leads. The drugs used to treat HIV fall into three categories: the nucleosides, non-nucleosides, and the protease inhibitors. The nucleoside and non-nucleoside inhibit the reverse transcriptase enzyme, while the third category of drugs inhibits the enzyme protease. These drugs are given in combinations of two or three to attack the HIV in different ways.

See also AIDS, recent advances in research and treatment; Antibody and antigen; Antiviral drugs; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Infection and resistance; Public health, current issues; T cells or T lymphocytes; Virology; Virus replication; Viruses and responses to viral infection

HUMAN LEUKOCYTE ANTIGEN (HLA)

The human leukocyte **antigen** (HLA) is not a single antigen, but is rather a group of proteins that are located on the surface of white blood cells. These proteins have a pivotal role in the body's immune response to foreign material. Because the HLA is a chemical tag that distinguishes "self" from "non-self," the antigen is important in the rejection of transplanted tissue and in the development of certain diseases (e.g., insulin-dependent diabetes).

The HLA is the human version of a complex that is known as the **major histocompatibility complex**. Similar complexes exist in other species. Indeed much of the early knowledge of the antigen complex came from work on mice in the early decades of the twentieth century. Research on human blood cells in the 1950s identified three genes associated with the HLA (HLA-A, HLA-B, HLA-C). In the 1970s, another **gene** was identified (HLA-D). With the advent of molecular technology beginning in the 1980s, more genes that code for proteins that function in the antigen complex have continued to be identified.

The HLA evolved to serve two functions. The first is to chemically label a cell in a manner that is unique to that cell. White blood cells from all but an identical twin will have differently structured HLAs on their surface. Thus, if white blood cells from one person are injected into someone else, the injected cells will be recognized as foreign. This recognition occurs because the HLA groups are "read" by an immune cell called the T cell. Essentially the different HLA arrangement on cells allows the **immune system** to develop an inventory of "self" antigens in the body. Knowing the "self" antigen allows the immune system to rapidly distinguish foreign antigens.

HLAs are a class of what is referred to as the major **histocompatibility** complex. These molecules are made up of a portion that is embedded in the cell membrane and a portion that protrudes out from the membrane's outer surface. The molecules function to identify a cell to the T lymphocyte cells of the immune system. The T cell will recognize a region of the histocompatibility complex as a host structure, and no immune reaction will be initiated towards the cell. In another host, the same region could be recognized as foreign by the T lymphocytes.

HLA-D is a so-called class II major histocompatibility molecule. Class II molecules have two segments that are embedded in the membrane. At the outer surface of the cell the molecule contains an antigen that has been acquired from the surrounding environment. Particles are engulfed, broken down into their constituent parts, and some of the components end up incorporated into the class II histocompatibility complex. Thus phenomenon is referred to as antigen presentation.

Class II molecules are not present on all cells the way class I molecules are. Rather, class II molecules are on the surface of immune cells such as macrophages and B-lymphocytes that are designed to process cells and present the antigens from these cells to T lymphocytes. This is done to increase the repertoire of antibodies that an organism possesses.

The two classes of histocompatibility molecules allow an organism to in essence establish an inventory of what cells are "self" and to expose foreign antigens to the immune system so that antibodies to these antigens can be made. In the future, an invading organism that possesses one or some of these "non-self" antigens will be swiftly recognized as an invader and will be dealt with.

Defects in the structure of the HLAs is the cause of some diseases where the body's immune system perceives a host antigen as foreign and begins to attack the body's own tissue. An example is insulin-dependent diabetes, where a host immune response causes the destruction of insulin producing cells.

See also Histocompatibility; Immune system; Immunodeficiency diseases

HUMAN T-CELL LEUKEMIA VIRUS (HTLV)

Two types of human T-cell Leukemia Virus (HTLV) are known. They are also known as human T-cell lymphotrophic **viruses**. HTLV-1 often is carried by a person with no overt symptoms being apparent. However, HTLV-I is capable of causing a number of maladies. These include abnormalities of the **T cells** and **B cells**, a chronic infection of the myelin covering of nerves that causes a degeneration of the nervous system, sores on the skin, and an **inflammation** of the inside of the eye. HTLV-II infection usually does not produce any symptoms. However, in some people a cancer of the blood known as hairy cell leukemia can develop.

At one time there was a third HTLV virus. However, what once called HTLV-III is now referred to as the **Human Immunodeficiency Virus** (**HIV**). HIV is generally accepted to be the causative agent of acquired **immunodeficiency** syndrome.

HTLV is a type of virus called a retrovirus. These viruses are unique in that they possess an enzyme that enables them to manufacture **deoxyribonucleic acid** from their constituent **ribonucleic acid**.

HTLV-I is most commonly associated with a disease called adult T-cell leukemia, which is a rapidly spreading cancerous growth that affects the T cells of the **immune system**. Indeed, the virus was first isolated in 1980 from a patient with T-cell lymphoma. Once the symptoms of the disease appear, deterioration of the individual occurs quickly. However, the symptoms may not appear for decades after the virus has infected someone. The reason for this extended period of latency is not known. HTLV-I has also been isolated from people who have maladies that include arthritis, Kaposi's sarcoma, and non-Hodgkin's lymphoma. Whether the virus is a contributor to such maladies, or is coincidentally expressed, is not yet clear.

The HTLV-I form of the virus is found all over the world. However, it is more prevalent in some countries, such as Japan, than in other countries, such as the United States.

HTLV-II was isolated in 1982 from a patient with hairy cell leukemia. Even so, the virus still has not been definitively established as the cause of that malady. However, the frequent isolation of HTLV-II from patients with this form of leukemia, as well as other types of leukemia's and lymphomas, lends credence to the theory that the virus is vital for the development of the malignancies.

HTLV-II is found in many intravenous drug users. Transmission of the virus from person to person via the contaminated blood in the needles used for drug injection has been documented. HTLV-II can also be spread by exchange of other body fluids, such as occurs in sexual contact.

In spite of the above conditions associated with HTLV-II, the majority of those infected with the virus do not display any symptoms.

The virus can be passed from person to person by the transfer of contaminated blood or via the intimate association of sexual contact. Also, the virus is capable of being passed from mother to infant via breast milk. Blood donor programs in many countries now rigorously test for the presence of HTLV in donated blood and plasma.

HTLV infections are incurable. However, the progressive physical deterioration associated with the infections can be lessened somewhat if the infections are diagnosed early. Screening for the virus relies on the detection of antibodies. Typically, antigen-antibody agglutination tests or the **enzyme-linked immunosorbent assay** (**ELISA**) is used. Confirmation of infections is provided by demonstrating the presence of viral protein in electrophoretic gels following the application of an **antibody** (the technique is dubbed the Western Blot).

See also AIDS; Immunodeficiency

HUMORAL IMMUNE RESPONSE • *see*

IMMUNITY, HUMORAL REGULATION

HYDROPHOBIC AND HYDROPHILIC

Hydrophobic and hydrophilic forces are interactions that serve to keep chemical groups positioned close to one another. Such associations are vital for the structure of the components of **microorganisms**.

Hydrophobic ("water hating") interactions are created because of the uncharged nature of the involved chemical groups. An example of such a chemical group is CH_3. All the bonds around the carbon atom are occupied. The chemical group is described as being nonpolar. Thus, a water molecule—a polar molecule—is unable to establish an association with the non-polar chemical group. This tends to create instability in the network of water molecules, and so is undesirable. The repulsive force of the surrounding water molecules acts to force hydrophobic regions into an association with like regions. The effect tends to be the formation of a hydrophobic "pocket" or "envelope" in a protein or a carbohydrate molecule or matrix.

Hydrophilic ("water loving) interactions are possible with polar chemical group. Water is polar because oxygen is far more electronegative than hydrogen and thus the electrons involved in an oxygen-hydrogen bond spend more time in proximity to the oxygen atom. Because of this unequal electron sharing, the oxygen atom takes on a partial negative charge and the hydrogen atom a partial positive charge. In addition, the bonds in a water molecule (oriented at 105° in a "bent" molecular shape) cannot cancel each other out. Other polar groups can then form ionic type bonds with water. Regions of proteins and other biological materials that are exposed to the environment are typically hydrophilic.

Hydrophobic and hydrophilic interactions can affect protein shape. Because of the polar or nonpolar nature of the constituent amino acid building blocks, as well as in carbohydrate and lipid constituents of microorganisms, molecules and sometimes whole microorganisms can assume shapes and orientations that depend on the intracellular or extracellular environment.

"Black smoker" hydrothermal vent, with tubes of worms growing to the right.

The tendency for hydrophobic regions of a protein of a lipid molecule to associate away from water is a main driving force in the folding of proteins into their dimensional configuration. Furthermore, the formation of biological membranes would be extremely difficult in the absence of hydrophobic and hydrophilic interactions. The biological molecules known as **phospholipids** have a hydrophilic "head" region and a nonpolar, hydrophobic "tail." These forces cause the phospholipid molecules to aggregate together so that the polar heads are oriented towards the water and the hydrophobic tails are buried inside. The effect is to spontaneously establish a membrane. Insertion of functionally specialized proteins into this so-called phospholipid bilayer acts to create a biological membrane of great complexity.

See also Bacterial membranes and cell wall; Biochemical analysis techniques; Biochemistry; Cell membrane transport; Membrane fluidity

HYDROTHERMAL VENTS

A hydrothermal vent is a geyser that is located on the floor of the sea. The first such vent was discovered in 1977 on the floor of the Pacific Ocean. Since then, vents have been discovered at a variety of locations in the Pacific and Atlantic Oceans.

The vents tend to be located deep in the ocean. For example, in the Atlantic ocean, some 7000 feet beneath the surface, hydrothermal vents are associated with underwater mountain chain called the Mid-Ocean Ridge. This ridge is geologically active with an upwelling of hot magma and volcanic activity. The tectonic plate movements cause faulting and seawater that then enters the cracks is superheated by the molten magma. The superheated water and steam and spews out through hydrothermal vents.

Some vents, known as "black smokers," spew out a black-colored mixture of iron and sulfide. "White smokers" spew out a whitish mix of barium, calcium, and silicon.

This eruption through the hydrothermal vents is continuous, in contrast with the sporadic eruptions of surface gey-

sers. The material that emerges from hydrothermal vents is extremely hot (up to 750° F [398.89° C]) and is very rich in minerals such as sulfur. The minerals can precipitate out of solution to form chimneys. The construction of a chimney can occur quickly. Growth of 30 feet in 18 months is not unusual. The tallest of these chimneys that has been measured was the height of a 15 story building.

A vibrant community of **bacteria**, tubeworms that are unique to this environment, and other creatures exists around hydrothermal vents. The entire ecosystem is possible because of the activity of the bacteria. These bacteria have been shown, principally through the efforts of the **Holger Jannasch** (1927–1998) of Woods Hole Oceanographic Institution, to accomplish the conversion of sulfur to energy in a process that does not utilize sunlight called chemosynthesis. The energy is then available for use by the other life forms, which directly utilize the energy, consume the bacteria, or consume the organisms that rely directly on the bacteria for nourishment. For example, the tubeworms have no means with which to take in or process nutrients. Their existence relies entirely on the bacteria that live in their tissues.

See also Chemoautotrophic and chemolithotrophic bacteria; Extremophiles; Sulfur cycle in microorganisms

HYGIENE

Hygiene refers to the health practices that minimize the spread of infectious **microorganisms** between people or between other living things and people. Inanimate objects and surfaces, such as contaminated cutlery or a cutting board, may be a secondary part of this process.

One of the bedrock fundamentals of hygiene is handwashing. The recognition of the link between handwashing and reduction in microbial illness dates back to the mid-nineteenth century. Then, Florence Nightingale, based on her nursing experiences during the Crimean War, wrote about her perceived relationship between unsanitary conditions and disease. At about the same time, the Viennese physician **Ignaz Philipp Semmelweis** noted the connection between mortalities in hospital patients and the movement of physicians from patient to patient without an intervening washing of their hands. After Semmelweis introduced hand washing with a solution containing chloride, the incidence of mortality due to puerperal fever (infection after childbirth) diminished from 18% to less than 3%. Now, hand washing with similar antiseptic agents and even with plain soap and water is known to reduce illness and death from hospital acquired infections.

Proper hygienic practices in the hospital setting not only save lives, but save a great deal of money also. According to the **Centers for Disease Control**, the cost of dealing with the 2.4 million hospital acquired urinary tract infections, blood stream infections, respiratory infections and infections of incisions, which are caused each year by microbes transferred from hospital staff to the patient, and which could be prevented by proper hand washing, is over 4 billion dollars in the United States alone.

Hand washing is a means of preventing the spread of bacterial and viral infections.

Similarly, in the home and other social settings, hand washing can prevent the spread of infectious microorganisms. A common route of infection is from the bathroom to the kitchen. Improper hand washing fails to remove microorganisms, such as ***Escherichia coli*** and *Salmonella* acquired from fecal excretion. Handling of food transfers the organisms to the food.

Hygiene is not so concerned with the bacterial flora that normally resides on the skin. These **bacteria** include *Corynebacterium*, *Proprionibacterium*, and *Acinetobacter*. These organisms are tenaciously associated with the skin and so are not as easily removed by the mechanical scrubbing action of hand washing. Rather, hygienic efforts such as hand washing attempt to remove organisms, such as *Escherichia coli* that become transiently associated with the skin. The transient organisms tend be removed more easily and are more apt to be infectious.

In medical environments, hygiene is not only mandatory, but must be done according to established procedures. For example, both before and after seeing a patient, a physician must wash his/her hands with an alcohol-based preparation if hands are not visibly dirty, and with soap and water if dirt is apparent. This practice is also done if any contact with microorganisms has occurred or is even suspected of occurring (for example, handling a surgical instrument that is not wrapped in a sterile package) and after removing surgical gloves. The latter is important since the interior of a surgical

glove can be an ideal breeding ground for bacteria. Furthermore, the act of handwashing is to be done for a specified period of time and with vigorous rubbing together of the hands and fingers. This is because the removal of microorganisms is accomplished not only by the presence of the soap but also by the friction of the opposing skin surfaces rubbing together.

Other hygienic practices in a laboratory include wiping the lab bench with a disinfectant compound before and after use and keeping the work area orderly and free of debris. Protective clothing can also be worn to minimize the spread of microorganisms. Such clothing includes hair nets, disposal boots and gloves, and lab coats. These items are worn in the vicinity of the work bench or other areas where microorganisms are expected, but are removed when exiting such locations.

Mechanical aids to hygiene exist. For example, many labs contain a **fume hood**, in which airflow is directed inward. Such laminar flow hoods do not allow the contaminated air inside the hood to move outward into the laboratory. Another standard feature of a microbiological laboratory is a small flame source. The flame is used to sterilize the lip of test tubes and vials before and after opening the containers, and to heat-sterilize the metal inoculating loops used to transfer microorganisms from one place to another.

While necessary for the protection of patients and to prevent **contamination** in the laboratory, the use of hygienic substances can have adverse effects. In the late 1980s the so-called "hygiene hypothesis" proposed that the increased use of disinfectants, particularly in the home, had decrease the exposure of people to substances that stimulated their **immune system**, and so had rendered the immune system less capable of dealing with environmental antigens. The result was proposed to be an increase in **allergies**. Time has strengthened this hypothesis to the point where the overuse of disinfectants has become a legitimate concern.

In addition to the development of allergic reactions, the inadequate or improper use of a hygienic compound can select for organisms that are more capable of causing disease. For example, certain disinfectants containing the compound triclosan have been shown to not only fail to kill the entire target *Escherichia coli* population, but to actually stimulate the development of resistance in those microbes that survive. In a setting such as a kitchen, the results could be problematic.

See also Acne, microbial basis of; Antiseptics; Disinfection and disinfectants; Transmission of pathogens

HYPHAE

One of the biological characteristics that distinguish multicellular **fungi** from other organisms is their constitutional cells, or hyphae (singular, hypha). Hyphae are nucleated cells in the shape of thin tubes, externally enveloped in a rigid chitin-rich cell wall and presenting an internal plasmatic membrane. They contain cellular organelles such as mitochondria, Golgi apparatus, **ribosomes**, endoplasmatic reticulum, which is also found in other **Eukaryotes** as well as cytoplasmatic vesicles bound to the plasmatic membrane. Hyphal cytoskeleton is organized by microtubules. Hyphae are separated by walls termed septae (singular, septum), usually bearing pores and regulatory structures that prevent cellular leaking due to cell disruption. For instance, the septum of Ascomycetes contains the Woronin body, an oily structure that blocks the pore if cell disruption occurs, whereas **Basidomycetes** have a dolipore septum, with the hyphae containing distinctly layered wall structures and endoplasmatic reticulum next to the pore. Hyphal growth and proliferation form structures similar to fine branches, which form the **mycelium** or vegetative hyphal network. However, Zygomycetes and Chytridiomycetes have non-septate vegetative mycelium, except for the reproductive structures.

As fungi grow, the older layers of hyphae gradually die because growth occurs through the proliferation and branching of the apical cell of the mycelium (i.e., cell at the mycelium tip). Growth takes place when two cytoplasmatic vesicles bound to the internal membrane fuse at the apical hypha, enlarging the hyphal tip because of the accumulation of biomass, leading to septum formation and branching. Branching is due to the growth of another apical cell inside the sub apical region of the mother cell. The growth process so far described is directed into new regions of the organic substrate, from which the fungus is feeding, and is termed the extension zone. The aerial part of the fungus, consisted of older hyphae forming aerial mycelia, may develop and differentiate to form structures bearing spores, and is termed the productive zone.

For most fungi, the haploid spore is the starting point from which the haploid hypha will develop and form the monokaryotic mycelium. The joining of two haploid mycelia leads to diploidization of the apical cells, resulting in two cells containing two separate nuclei, known as a dikaryotic mycelium. These cells are capable of producing spores and in Basidomycetes, are termed basidia (singular, basidium) whereas in Ascomycetes, they are termed ascus. Usually, a typical basidium produces four sexual spores and the ascus can produce eight spores, although the amount of spores can vary among the species of a given phylum. In the basidium, for instance, the two nuclei are duplicated and then merged when the cell is about to undergo meiosis twice, thus resulting in the formation of four haploid spores. Some Ascomycetes as well as a few Basidomycetes may also produce asexual spores; and asexual reproduction is the way Zygomycetes and Chytridiomycetes reproduce themselves.

See also Fungal genetics; Fungi; Mycology; Parasites; Yeast

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IgA • *see* IMMUNOGLOBULINS AND IMMUNOGLOBULIN DEFICIENCY SYNDROMES

IgA DEFICIENCY • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

IgD • *see* IMMUNOGLOBULINS AND IMMUNOGLOBULIN DEFICIENCY SYNDROMES

IgE • *see* IMMUNOGLOBULINS AND IMMUNOGLOBULIN DEFICIENCY SYNDROMES

IgG • *see* IMMUNOGLOBULINS AND IMMUNOGLOBULIN DEFICIENCY SYNDROMES

IgG SUBCLASS AND SPECIFIC ANTIBODY DEFICIENCIES • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

IgM • *see* IMMUNOGLOBULINS AND IMMUNOGLOBULIN DEFICIENCY SYNDROMES

IMMUNE COMPLEX TEST

The immune complex test is a test designed to evaluate the status or proper functioning of the **immune system**. The criterion used to evaluate the operation of the immune system is via the presence of so-called immune complexes.

An immune complex is an association formed between large numbers of antigens and the corresponding antibodies. The latter are produced in a specific response to the presence of the **antigen**, which has been perceived as being foreign by the body's immune system. The individual antigen-antibody complexes can associate together to form the interlocking network that represents an immune complex.

Normally, immune complexes are removed from the bloodstream by specialized cells of the spleen called macrophages and by other specialized cells located in the liver. However, if this clearance does not occur, the immune complexes will continue to circulate, and will become trapped in the kidneys, lung, skin, joints, or blood vessels. The specific location depends on the composition of the complex. Their presence will cause **inflammation** and can lead to tissue damage.

Immune complexes can develop as a result of what is termed a low-grade persistent infection. Examples include *Streptococcus viridans* infection of the blood, *Staphylococcus* heart infections, and viral **hepatitis**. Second, immune complexes can form in response to the continued exposure to an antigen, such as the repeated inhalation of **mold** in a farming or animal care facility. Finally, immune complexes are often a hallmark of autoimmune diseases. The continual response of the body's immune system overloads the ability of the body to remove the immune complexes that form. Examples of autoimmune diseases for which an immune test is beneficial in terms of diagnosis are systemic lupus erythematosus, rheumatoid arthritis, **Lyme disease** and **human immunodeficiency virus** infection.

Being able to test for the presence of abnormal levels of immune complexes can alert the physician to an abnormal function of the immune system, such as an autoimmune disorder.

Immune complexes can be detected by the application of special stains to tissue that has been obtained from a patient. The stains contain antibodies that bind to the complexes and this binding is highlighted by the presence of the staining agent. This test is useful because it directly detects the presence of the immune complexes. However, for routine clinical use, this method is cumbersome and invasive. This has stimulated the development of blood tests that indirectly detect the complexes in the blood serum.

There are several methods available. Often more than one will be used to test the same sample. This is because the

test methods are not yet uniformly standardized across the medical community. But, if the results from several tests are positive for immune complexes, the validity of the diagnosis is ensured.

Immune complex tests include the Raji cell, C1q binding, conglutinin, and anti-C3 assays. The Raji cell assay, for example, detects the immune complexes following the binding of the complexes with an immune molecule called **complement**. In addition, the complement has been labeled with a compound known as fluorescein isothiocyanate. The latter compound is able to fluoresce when light of a certain wavelength is shone on it. The detection occurs in a machine called a flow cytometer, in which fluid moves past a detector that is programmed to detect certain chemical aspects. In the Raji cell assay, detection of the fluorescent isothiocyanate indicates the presence of the immune complex.

A normal result in an immune complex test is a negative result. In other words, immune complexes are normally absent.

See also Antibody-antigen, biochemical and molecular reactions; Laboratory techniques in immunology

IMMUNE STIMULATION, AS A VACCINE

Immune stimulation refers to the stimulation of the **immune system** by an external source. The stimulation can confer a protective effect against **microorganisms**. As well, immune stimulation shows promise as a means of obtaining an immune response to conditions such as cancer.

Conventionally, the immune system is stimulated into producing antibodies or other infection-fighting constituents in response to an infection. Immune stimulation seeks to elicit the immune reaction before infection or other malady strikes, as a means of preventing the infection or malady. This approach is analogous to the administration of components of weakened or inactive **influenza** virus to protect people from the subsequent onset of influenza.

The roots of the use of immune stimulation as a **vaccine** date back to the late nineteenth century. Then, William Coley, a New York bone surgeon, began treatments in which he injected cancer sufferers with a preparation consisting of dissolved *Streptococcus pyogenes* **bacteria**. Anecdotal evidence claimed remission of tumor growth in 40% of the treated patients. Then, in the 1980s, it was discovered that the observed anti-tumor activity of a bacteria known as *Bacillus Calmette-Guerin* was a property of the construction of the bacterial genetic material. Indeed, the bacterial genetic material is able to stimulate the immune system such that the target sequence of the bacterial **gene** is distinguished from the host genetic material. The resulting immune stimulation boosts **antibody** levels as well as another aspect of the immune system known as cell-mediated **immunity**.

Synthetic peptides have also proved useful as agents of immune stimulation. These compounds are made up of chains of amino acids. They are called synthetic because they are not naturally occurring, but rather are constructed in the laboratory. The peptide can contain amino acids in which a chemical group is oriented in a mirror image of that which is normally found in nature.

The mirror image arrangement proves lethal to various bacteria. For example, synthetic peptides swiftly kill populations of *Staphylococcus aureus* and *Enterococcus faecium* that are resistant to an array of **antibiotics**. The peptide binds to the outer surface of the bacterium and is able to punch a hole through the cell wall. The punctured bacteria die. Furthermore, as the bacteria release their contents, the immune system is stimulated to produce antibodies to the bacterial constituents. The synthetic peptide both kills bacteria directly and stimulates an immune response that acts to kill more bacteria.

Thus far, immune stimulation as a vaccine has been developed towards so-called extracellular infections. These are infection caused by bacteria that adhere to host cells or that proliferate in fluids such as blood. For these types of infections, the immune stimulation aims to produce antibodies. Defense against intracellular infections, which are caused by bacteria invading host cells, requires the stimulation of other immune components, such as phagocytic cells. Furthermore, defense against viral infections requires stimulation of immune components called killer cells.

Synthetic peptides can also stimulate the immune system to recognize a surface constituent of a certain type of cancer cell called a melanoma cell. Melanoma is also commonly referred to as skin cancer. The synthetic peptide can mimic the peptide produced on a tumor that the immune system can recognize and respond to. By supplying the target externally, the immune system has more opportunity to mount a defense against the offending peptide. The resulting antibody molecules would target the peptide on the tumor cells.

While shrinkage of tumors was evident in laboratories studies, confirmation of the clinical power of the technique requires a clinical trial where many people are given the treatment and their progress monitored. Nonetheless, it has been demonstrated that synthetic peptides are capable of stimulating the ability of the immune system to distinguish between antigens that are an innate part of the body from those **antigen** that come from outside of the body. In the case of many cancers, such immune stimulation is required, as the disease can compromise the natural immune defenses.

See also Immunization; Immunologic therapies

IMMUNE SYNAPSE

Before they can help other immune cells respond to a foreign protein or pathogenic organism, helper **T cells** must first become activated. This process occurs when an antigen-presenting cell submits a fragment of a foreign protein, bound to a Class II **MHC** molecule (virus-derived fragments are bound to Class I MHC molecules) to the helper T cell. Antigen-presenting cells are derived from bone marrow, and include both dendritic cells and Langerhans cells, as well as other specialized cells. Because T cell responses depend upon direct con-

tact with their target cells, their **antigen** receptors, unlike antibodies made by **B cells**, exist bound to the membrane only. In the intercellular gap between the T cell and the antigen-presenting cell, a special pattern of various receptors and complementary ligands forms that is several microns in size. This patterned collection of receptors is called the immune synapse.

The immune synapse can be compared to a molecular machine that controls T cell activation. Physically it consists of a group of T cell receptors surrounded by a ring of integrin-like adhesion molecules as well as other accessory proteins like the CD3 complex. Integrins are a family of cell-surface proteins that are involved in binding to extracellular matrix components. This specialized cell-cell junction was named the immunological synapse because it is thought to be involved in the transfer of information across the T cell-APC junction. Specifically, the immune synapse appears to play an essential role in organizing the immune response, the level of control, and the nature of that response. The formation of the synapse requires several minutes and it appears to be stable for several hours. The structural protein actin seems to have an important role in that stability as T-cell activation is blocked by disruption of actin filaments. There also appears to be a temporal spatial component in that signals that modulate T-cell maturity and functions are received in a serial manner as well as simultaneously. Further clarification of the structure of the immune synapse will help develop further insights into T cell recognition as well as the mechanism of T cell receptor signaling - how information transfer occurs across the synapse. The duration of signaling in immature T cells may control CD4 and CD8 lineage decisions. This would be useful in determining the degree to which different types and developmental stages rely on alternative signaling mechanisms.

See also Antibody and antigen; Antibody formation and kinetics; Antibody-antigen, biochemical and molecular reactions; T cells or T-lymphocytes

IMMUNE SYSTEM

The immune system is the body's biological defense mechanism that protects against foreign invaders. Only in the last century have the components of that system and the ways in which they work been discovered, and more remains to be clarified.

The true roots of the study of the immune system date from 1796 when an English physician, **Edward Jenner**, discovered a method of **smallpox vaccination**. He noted that dairy workers who contracted **cowpox** from milking infected cows were thereafter resistant to smallpox. In 1796, Jenner injected a young boy with material from a milkmaid who had an active case of cowpox. After the boy recovered from his own resulting cowpox, Jenner inoculated him with smallpox; the boy was immune. After Jenner published the results of this and other cases in 1798, the practice of Jennerian vaccination spread rapidly.

It was **Louis Pasteur** who established the cause of infectious diseases and the medical basis for **immunization**. First, Pasteur formulated his **germ theory of disease**, the concept that disease is caused by communicable **microorganisms**. In 1880, Pasteur discovered that aged cultures of fowl cholera **bacteria** lost their power to induce disease in chickens but still conferred **immunity** to the disease when injected. He went on to use attenuated (weakened) cultures of **anthrax** and **rabies** to vaccinate against those diseases. The American scientists Theobald Smith (1859–1934) and Daniel Salmon (1850–1914) showed in 1886 that bacteria killed by heat could also confer immunity.

Why vaccination imparted immunity was not yet known. In 1888, Pierre-Paul-Emile Roux (1853–1933) and Alexandre Yersin (1863–1943) showed that **diphtheria** bacillus produced a toxin that the body responded to by producing an antitoxin. **Emil von Behring** and **Shibasaburo Kitasato** found a similar toxin-antitoxin reaction in **tetanus** in 1890. Von Behring discovered that small doses of tetanus or diphtheria toxin produced immunity, and that this immunity could be transferred from animal to animal via serum. Von Behring concluded that the immunity was conferred by substances in the blood, which he called antitoxins, or antibodies. In 1894, Richard Pfeiffer (1858–1945) found that antibodies killed cholera bacteria (bacterioloysis). Hans Buchner (1850–1902) in 1893 discovered another important blood substance called **complement** (Buchner's term was alexin), and **Jules Bordet** in 1898 found that it enabled the antibodies to combine with antigens (foreign substances) and destroy or eliminate them. It became clear that each **antibody** acted only against a specific **antigen**. **Karl Landsteiner** was able to use this specific antigen-antibody reaction to distinguish the different blood groups.

A new element was introduced into the growing body of immune system knowledge during the 1880s by the Russian microbiologist Elie Metchnikoff. He discovered cell-based immunity: white blood cells (leucocytes), which Metchnikoff called phagocytes, ingested and destroyed foreign particles. Considerable controversy flourished between the proponents of cell-based and blood-based immunity until 1903, when **Almroth Edward Wright** brought them together by showing that certain blood substances were necessary for phagocytes to function as bacteria destroyers. A unifying theory of immunity was posited by **Paul Ehrlich** in the 1890s; his "side-chain" theory explained that antigens and antibodies combine chemically in fixed ways, like a key fits into a lock. Until this time, immune responses were seen as purely beneficial. In 1902, however, Charles Richet and Paul Portier demonstrated extreme immune reactions in test animals that had become sensitive to antigens by previous exposure. This phenomenon of hypersensitivity, called **anaphylaxis**, showed that immune responses could cause the body to damage itself. Hypersensitivity to antigens also explained **allergies**, a term coined by Pirquet in 1906.

Much more was learned about antibodies in the mid-twentieth century, including the fact that they are proteins of the gamma globulin portion of plasma and are produced by plasma cells; their molecular structure was also worked out. An important advance in **immunochemistry** came in 1935 when Michael Heidelberger and Edward Kendall (1886–1972) developed a method to detect and measure amounts of differ-

ent antigens and antibodies in serum. Immunobiology also advanced. **Frank Macfarlane Burnet** suggested that animals did not produce antibodies to substances they had encountered very early in life; Peter Medawar proved this idea in 1953 through experiments on mouse embryos.

In 1957, Burnet put forth his clonal **selection** theory to explain the biology of immune responses. On meeting an antigen, an immunologically responsive cell (shown by C. S. Gowans (1923–) in the 1960s to be a lymphocyte) responds by multiplying and producing an identical set of plasma cells, which in turn manufacture the specific antibody for that antigen. Further cellular research has shown that there are two types of lymphocytes (nondescript lymph cells): B-lymphocytes, which secrete antibody, and **T-lymphocytes**, which regulate the B-lymphocytes and also either kill foreign substances directly (killer **T cells**) or stimulate macrophages to do so (helper T cells). Lymphocytes recognize antigens by characteristics on the surface of the antigen-carrying molecules. Researchers in the 1980s uncovered many more intricate biological and chemical details of the immune system components and the ways in which they interact.

Knowledge about the immune system's role in rejection of transplanted tissue became extremely important as organ transplantation became surgically feasible. Peter Medawar's work in the 1940s showed that such rejection was an immune reaction to antigens on the foreign tissue. Donald Calne (1936–) showed in 1960 that immunosuppressive drugs, drugs that suppress immune responses, reduced transplant rejection, and these drugs were first used on human patients in 1962. In the 1940s, George Snell (1903–1996) discovered in mice a group of tissue-compatibility genes, the **MHC**, that played an important role in controlling acceptance or resistance to tissue grafts. Jean Dausset found human MHC, a set of antigens to human leucocytes (white blood cells), called **HLA**. Matching of HLA in donor and recipient tissue is an important technique to predict compatibility in transplants. Baruj Benacerraf in 1969 showed that an animal's ability to respond to an antigen was controlled by genes in the MHC complex.

Exciting new discoveries in the study of the immune system are on the horizon. Researchers are investigating the relation of HLA to disease; certain types of HLA molecules may predispose people to particular diseases. This promises to lead to more effective treatments and, in the long run, possible prevention. Autoimmune reaction, in which the body has an immune response to its own substances, may also be a cause of a number of diseases, like multiple sclerosis, and research proceeds on that front. Approaches to cancer treatment also involve the immune system. Some researchers, including Burnet, speculate that a failure of the immune system may be implicated in cancer. In the late 1960s, Ion Gresser (1928–) discovered that the protein interferon acts against cancerous tumors. After the development of genetically engineered interferon in the mid-1980s finally made the substance available in practical amounts, research into its use against cancer accelerated. The invention of monoclonal antibodies in the mid-1970s was a major breakthrough. Increasingly sophisticated knowledge about the workings of the immune system holds out the hope of finding an effective method to combat one of the most serious immune system disorders, **AIDS**.

Avenues of research to treat AIDS includes a focus on supporting and strengthening the immune system. (However, much research has to be done in this area to determine whether strengthening the immune system is beneficial or whether it may cause an increase in the number of infected cells.) One area of interest is **cytokines**, proteins produced by the body that help the immune system cells communicate with each other and activate them to fight infection. Some individuals infected with the AIDS virus **HIV** (**human immunodeficiency virus**) have higher levels of certain cytokines and lower levels of others. A possible approach to controlling infection would be to boost deficient levels of cytokines while depressing levels of cytokines that may be too abundant. Other research has found that HIV may also turn the immune system against itself by producing antibodies against its own cells.

Advances in immunological research indicate that the immune system may be made of more than 100 million highly specialized cells designed to combat specific antigens. While the task of identifying these cells and their functions may be daunting, headway is being made. By identifying these specific cells, researchers may be able to further advance another promising area of immunologic research, the use of recombinant **DNA** technology, in which specific proteins can be mass-produced. This approach has led to new cancer treatments that can stimulate the immune system by using synthetic versions of proteins released by **interferons**.

See also Antibody and antigen; Antibody formation and kinetics; Antibody, monoclonal; Antibody-antigen, biochemical and molecular reactions; B cells or B lymphocytes; Bacteria and bacterial infection; Germ theory of disease; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunochemistry; Immunodeficiency; Immunogenetics; Immunologic therapies; Immunological analysis techniques; Immunology, nutritional aspects; Immunology; Immunosuppressant drugs; Infection and resistance; Invasiveness and intracellular infection; Major histocompatibility complex (MHC); T cells or T-lymphocytes; Transmission of pathogens; Transplantation genetics and immunology; Viruses and responses to viral infection

IMMUNITY: ACTIVE, PASSIVE, AND DELAYED

Active, passive, and delayed immunity are all variations on the operation of the **immune system**, whereby antibodies are produced in response to the presence of an **antigen** considered to be foreign.

Active immunity occurs due to the production of an **antibody** as a result of the presence of the target antigen either as part of an intact infecting organism, or because of the introduction of the specific antigen in the form of a **vaccine**. The immunity is provided by an individual's own immune system.

Vaccination against hepatitis.

The type of immunity invoked by the active response tends to be permanent. Once the antibody has been produced, an individual will be protected against the presence of the target antigen for a lifetime. The immune system has a capacity for memory of the antigen. If presented with the antigen challenge again, the immune machinery responsible for the formation of the corresponding antibody is rapidly triggered into action.

An example of active immunity is the injection into healthy individuals of the disabled toxins of **bacteria** such as *Corynebacterium diphtheriae*, the agent causing **diphtheria**, and *Clostridium tetani*, the agent that causes **tetanus**. This rational was first proposed by **Paul Ehrlich**. In 1927, Gaston Ramon attempted his suggestion. He separately injected inactivated version of the bacterial toxins and was able to demonstrate an immune response to both toxins. This rationale has carried forward to the present day. A combination vaccine containing both inactivated toxins is a routine inoculation in childhood.

Another historical development associated with active immunity involved **Louis Pasteur**. In 1884, Pasteur used weakened cultures of *Bacillus anthracis*, the causative agent of **anthrax**, and inactivated sample from the spinal cords of rabbits infected with the **rabies** virus to produce immunity to anthrax and rabies. Pasteur's method spurred the development of other active immune protective vaccines. Just one example is the oral **poliomyelitis** vaccine developed by **Albert Sabin** in the 1950s.

Passive immunity also results in the presence of antibody. However, the particular individual does not produce the antibody. Rather, the antibody, which has been produced in someone else, is introduced to the recipient. An example is the transfer of antibodies from a mother to her unborn child in the womb. Such antibodies confer some immune protection to the child in the first six months following birth. Indeed, the transient nature of the protection is a hallmark of passive immunity. Protection fades over the course of weeks or a few months following the introduction of the particular antibody. For example, a newborn carries protective maternal antibodies to several diseases, including **measles**, **mumps** and rubella. But by the end of the individual's first year of life, **vaccination** with the MMR vaccine is necessary to maintain the protection.

Another example of passive **immunization** is the administration to humans of tetanus antitoxin that is produced in a

horse in response to the inactivated tetanus toxin. This procedure is typically done if someone has been exposed to a situation where the possibility of contracting tetanus exists. Rather than rely on the individual's immune system to respond to the presence of the toxin, neutralizing antibodies are administered right away.

Active and passive immunity are versions of what is known as antibody-mediated immunity. That is, antibodies bind to the antigen and this binding further stimulates the immune system to respond to the antigen threat. Antibody-mediated immunity is also called humoral immunity.

A third type of immunity, which is known as delayed immunity or delayed-type hypersensitivity, is represents a different sort of immunity. Delayed immunity is a so-called cell-mediated immunity. Here, immune components called T-cells bind to the surface of other cells that contain the antigen on their surface. This binding triggers a further response by the immune system to the foreign antigen. The response can involve components such as white blood cells.

An example of delayed immunity is the tuberculin test (or the Mantoux test), which tests for the presence of *Mycobacterium tuberculosis*, the bacterium that causes **tuberculosis**. A small amount of bacterial protein is injected into the skin. If the individual is infected with the bacteria, or has ever been infected, the injection site becomes inflamed within 24 hours. The response is delayed in time, relative to the immediate response of antibody-based immunity. Hence, the name of the immunity.

See also Antibody formation and kinetics; Immunization

IMMUNITY, CELL MEDIATED

The **immune system** is a network of cells and organs that work together to protect the body from infectious organisms. Many different types of organisms such as **bacteria**, **viruses**, **fungi**, and **parasites** are capable of entering the human body and causing disease. It is the immune system's job to recognize these agents as foreign and destroy them.

The immune system can respond to the presence of a foreign agent in one of two ways. It can either produce soluble proteins called antibodies, which can bind to the foreign agent and mark them for destruction by other cells. This type of response is called a humoral response or an **antibody** response. Alternately, the immune system can mount a cell-mediated immune response. This involves the production of special cells that can react with the foreign agent. The reacting cell can either destroy the foreign agents, or it can secrete chemical signals that will activate other cells to destroy the foreign agent.

During the 1960s, it was discovered that different types of cells mediate the two major classes of immune responses. The T lymphocytes, which are the main effectors of the cell-mediated response, mature in the thymus, thus the name T cell. The **B cells**, which develop in the adult bone marrow, are responsible for producing antibodies. There are several different types of **T cells** performing different functions. These diverse responses of the different T cells are collectively called the "cell-mediated immune responses."

There are several steps involved in the cell-mediated response. The pathogen (bacteria, virus, fungi, or a parasite), or foreign agent, enters the body through the blood stream, different tissues, or the respiratory tract. Once inside the body, the foreign agents are carried to the spleen, lymph nodes, or the mucus-associated lymphoid tissue (MALT) where they will come in contact with specialized cells known as antigen-presenting cells (APC). When the foreign agent encounters the antigen-presenting cells, an immune response is triggered. These **antigen** presenting cells digest the engulfed material, and display it on their surface complexed with certain other proteins known as the Major **Histocompatibility** Class (**MHC**) of proteins.

Next, the T cells must recognize the antigen. Specialized receptors found on some T cells are capable of recognizing the MHC-antigen complexes as foreign and binding to them. Each T cell has a different receptor in the cell membrane that is capable of binding a specific antigen. Once the T cell receptor binds to the antigen, it is stimulated to divide and produce large amounts of identical cells that are specific for that particular foreign antigen. The T lymphocytes also secrete various chemicals (**cytokines**) that can stimulate this proliferation. The cytokines are also capable of amplifying the immune defense functions that can eventually destroy and remove the antigen.

In cell-mediated immunity, a subclass of the T cells mature into cytotoxic T cells that can kill cells having the foreign antigen on their surface, such as virus-infected cells, bacterial-infected cells, and tumor cells. Another subclass of T cells called helper T cells activates the B cells to produce antibodies that can react with the original antigen. A third group of T cells called the suppressor T cells is responsible for regulating the immune response by turning it on only in response to an antigen and turning it off once the antigen has been removed.

Some of the B and T lymphocytes become "memory cells," that are capable of remembering the original antigen. If that same antigen enters the body again while the memory cells are present, the response against it will be rapid and heightened. This is the reason the body develops permanent immunity to an infectious disease after being exposed to it. This is also the principle behind **immunization**.

See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Antibody, monoclonal; Antigenic mimicry; Immune stimulation, as a vaccine; Immune synapse; Immune system; Immunity, active, passive and delayed; Immunity, humoral regulation; Immunization; Immunochemistry

IMMUNITY, HUMORAL REGULATION

One way in which the **immune system** responds to pathogens is by producing soluble proteins called antibodies. This is known as the humoral response and involves the activation of a special set of cells known as the **B lymphocytes**, because

they originate in the bone marrow. The humoral immune response helps in the control and removal of pathogens such as **bacteria**, **viruses**, **fungi**, and **parasites** before they enter host cells. The antibodies produced by the **B cells** are the mediators of this response.

The antibodies form a family of plasma proteins referred to as **immunoglobulins**. They perform two major functions. One function of an **antibody** is to bind specifically to the molecules of the foreign agent that triggered the immune response. A second antibody function is to attract other cells and molecules to destroy the pathogen after the antibody molecule is bound to it.

When a foreign agent enters the body, it is engulfed by the antigen-presenting cells, or the B cells. The B cell that has a receptor (surface immunoglobulin) on its membrane that corresponds to the shape of the **antigen** binds to it and engulfs it. Within the B cell, the antigen-antibody pair is partially digested, bound to a special class of proteins called MHC-II, and then displayed on the surface of the B cell. The helper **T cells** recognize the pathogen bound to the MHC-II protein as foreign and becomes activated.

These stimulated T cells then release certain chemicals known as **cytokines** (or lymphokines) that act upon the primed B cells (B cells that have already seen the antigen). The B cells are induced to proliferate and produce several identical cells capable of producing the same antibody. The cytokines also signal the B cells to mature into antibody producing cells. The activated B cells first develop into lymphoblasts and then become plasma cells, which are essentially antibody producing factories. A subclass of B cells does not differentiate into plasma cells. Instead, they become memory cells that are capable of producing antibodies at a low rate. These cells remain in the immune system for a long time, so that the body can respond quickly if it encounters the same antigen again.

The antibody destroys the pathogen in three different ways. In neutralization, the antibodies bind to the bacteria or toxin and prevent it from binding and gaining entry to a host cell. Neutralization leads to a second process called **opsonization**. Once the antibody is bound to the pathogen, certain other cells called macrophages engulf these cells and destroy them. This process is called **phagocytosis**. Alternately, the immunoglobulin IgM or IgG can bind to the surface of the pathogen and activate a class of serum proteins called the **complement**, which can cause lysis of the cells bearing that particular antigen.

In the humoral immune response, each B cell produces a distinct antibody molecule. There are over a million different B lymphocytes in each individual, which are capable of recognizing a corresponding million different antigens. Since each antibody molecule is composed of two different proteins (the light chain and the heavy chain), it can bind two different antigens at the same time.

See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Immune system; Immunity, active, passive and delayed; Immunity, cell mediated

IMMUNIZATION

When a foreign disease-causing agent (pathogen) enters the body, a protective system known as the **immune system** comes into play. This system consists of a complex network of organs and cells that can recognize the pathogen and mount an immune response against it.

Any substance capable of generating an immune response is called an **antigen** or an immunogen. Antigens are not the foreign **bacteria** or **viruses** themselves; they are substances such as toxins or **enzymes** that are produced by the microorganism. In a typical immune response, certain cells known as the antigen-presenting cells trap the antigen and present it to the immune cells (lymphocytes). The lymphocytes that have receptors specific for that antigen binds to it. The process of binding to the antigen activates the lymphocytes and they secrete a variety of **cytokines** that promotes the growth and maturation of other immune cells such as cytotoxic T lymphocytes. The cytokines also act on **B cells** stimulating them to divide and transform into **antibody** secreting cells. The foreign agent is then either killed by the cytotoxic **T cells** or neutralized by the antibodies.

The process of inducing an immune response is called immunization. It may be either natural, i.e., acquired after infection by a pathogen, or, the **immunity** may be artificially acquired with serum or vaccines.

In order to make vaccines for immunization, the organism, or the poisonous toxins of the microorganism that can cause diseases, are weakened or killed. These vaccines are injected into the body or are taken orally. The body reacts to the presence of the **vaccine** (foreign agent) by making antibodies. This is known as active immunity. The antibodies accumulate and stay in the system for a very long time, sometimes for a lifetime. When antibodies from an actively immunized individual are transferred to a second non-immune subject, it is referred to as passive immunity. Active immunity is longer lasting than passive immunity because the memory cells remain in the body for an extended time period.

Immunizations are the most powerful and cost-effective way to prevent infectious disease in children. Because they have received antibodies from their mother's blood, babies are immune to many diseases when they are born. However, this immunity wanes during the first year of life. Immunization programs, therefore, are begun during the first year of life.

Each year in the United States, thousands of adults die needlessly from vaccine-preventable diseases or their complications. Eight childhood diseases (**measles**, **mumps**, rubella, **diphtheria**, **tetanus**, **pertussis**, *Hemophilus influenzae* type b, and polio) are preventable by immunization. With the exception of tetanus, all the other diseases are contagious and could spread rapidly, resulting in **epidemics** in an unvaccinated population. Hence, vaccinations are among the safest and most cost-efficient **public health** measures. Vaccinations against flu (**influenza**), **hepatitis** A, and pneumococcal disease are also recommended for some adolescents and adults. The vaccines indicated for adults will vary depending on lifestyle factors, occupation, chronic medical conditions and travel plans.

See also Antibody and antigen; Antibody formation and kinetics; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation

IMMUNOCHEMISTRY

Immunochemistry is the study of the chemistry of immune responses.

An immune response is a reaction caused by the invasion of the body by an **antigen**. An antigen is a foreign substance that enters the body and stimulates various defensive responses. The cells mainly involved in this response are macrophages and T and **B lymphocytes**. A macrophage is a large, modified white blood cell. Before an antigen can stimulate an immune response, it must first interact with a macrophage. The macrophage engulfs the antigen and transports it to the surface of the lymphocytes. The macrophage (or neutrophil) is attracted to the antigen by chemicals that the antigen releases. The macrophage recognizes these chemicals as alien to the host body. The local cells around the infection will also release chemicals to attract the macrophages; this is a process known as chemotaxis. These chemicals are a response to the infection. This process of engulfing the foreign body is called **phagocytosis**, and it leads directly to painful swelling and **inflammation** of the infected area.

Lymphocytes are also cells that have been derived from white blood cells (leucocytes). Lymphocytes are found in lymph nodes, the spleen, the thymus, bone marrow, and circulating in the blood plasma. Those lymphocytes that mature inside mammalian bone marrow are called **B cells**. Once B cells have come into contact with an antigen, they proliferate and differentiate into **antibody** secreting cells. An antibody is any protein that is released in the body in direct response to infection by an antigen. Those lymphocytes that are formed inside the thymus are called T lymphocytes or **T cells**. After contact with an antigen, T cells secrete lymphokines—a group of proteins that do not interact with the antigens themselves, instead they stimulate the activity of other cells. Lymphokines are able to gather uncommitted T cells to the site of infection. They are also responsible for keeping T cells and macrophages at the site of infection. Lymphokines also amplify the number of activated T cells, stimulate the production of more lymphokines, and kill infected cells. There are several types of T cells. These other types include T helper cells that help B cells mature into antibody-secreting cells, T suppresser cells that halt the action of B and T cells, T cytotoxic cells that attack infected or abnormal cells, and T delayed hypersensitivity cells that react to any problems caused by the initial infection once it has disappeared. This latter group of cells are long lived and will rapidly attack any remaining antigens that have not been destroyed in the major first stages of infection.

Once the antibodies are released by the B and T cells, they interact with the antigen to attempt to neutralize it. Some antibodies act by causing the antigens to stick together; this is a process known as agglutination. Antibodies may also cause the antigens to fall apart, a process known as cell lysis. Lysis is caused by **enzymes** known as lytic enzymes that are secreted by the antibodies. Once an antigen has been lysed, the remains of the antigen are removed by phagocytosis. Some antigens are still able to elicit a response even if only a small part of the antigen remains intact. Sometimes the same antibody will cause agglutination and then lysis. Some antibodies are antitoxins, which directly neutralize any toxins secreted by the antigens. There are several different forms of antibody that carry out this process depending upon the type of toxin that is produced.

Once antibodies have been produced for a particular antigen they tend to remain in the body. This provides **immunity**. Sometimes immunity is long term and once exposed to a disease we will never catch the disease again. At other times, immunity may only be short lived. The process of active immunity is when the body produces its own antibodies to confer immunity. Active immunity occurs after an initial exposure to the antigen. Passive immunity is where antibodies are passed form mother to child through the placenta. This form of immunity is short lived. Artificial immunity can be conferred by the action of **immunization**. With immunization, a **vaccine** is injected into the body. The vaccine may be a small quantity of antigen, it may be a related antigen that causes a less serious form of the disease, it may be a fragment of the antigen, or it may be the whole antigen after it has been inactivated. If a fragment of antigen is used as a vaccine, it must be sufficient to elicit an appropriate response from the body. Quite often viral coat proteins are used for this. The first vaccine was developed by **Edward Jenner** (1749–1823) in 1796 to inoculate against **smallpox**. Jenner used the mild disease **cowpox** to confer immunity for the potentially fatal but biochemically similar smallpox.

Within the blood there are a group of blood serum proteins called **complement**. These proteins become activated by antigen antibody reactions. Immunoglobulin is an antibody secreted by lymphoid cells called plasma cells. **Immunoglobulins** are made of two long polypeptide chains and two short polypeptide chains. These chains are bound together in a Y-shaped arrangement, with the short chains forming the inner parts of the Y. Each arm of the Y has specific antigen binding properties. There are five different classes of immunoglobulin that are based on their antigen-binding properties. Different classes of immunoglobulins come into play at different stages of infection. Immunoglobulins have specific binding sites with antigens.

One class of compounds in animals has antigens that can be problematical. This is the group called the **histocompatibility** complex. This is the group of usually surface proteins that are responsible for rejections and incompatibilities in organ transplants. These antigens are genetically encoded and they are present on the surface of cells. If the cells or tissues are transferred from one organism to another or the body does not recognize the antigens, it will elicit a response to try to rid the body of the foreign tissue. A body is not interested where foreign proteins come from. It is interested in the fact that they are there when they should not be. Even if an organ is human in origin, it must be genetically similar to the host body or it will be rejected. Because an organ is much larger than a small infection of an antigen when it elicits an immune response, it can be a greater problem. With an organ trans-

plant, there can be a massive cascade reaction of antibody production. This will include all of the immune responses of which the body is capable. Such a massive response can overload the system and it can cause death. Thus, tissue matching in organ transplants is vitally important. Often, a large range of immunosuppressor drugs are employed until the body integrates a particular organ. In some cases, this may necessitate a course of drugs for the rest of the individuals life. Histocompatibility problems also exist with blood. Fortunately, the proteins in blood are less specific and blood transfusions are a lot easier to perform than organ transplants. The blood-typing systems that are in use are indications of the proteins that are present. If blood is mixed from the wrong types, it can cause lethal clotting. The main blood types are A, B, O, and AB. Group O individuals are universal donors, they can give blood to anyone. Group AB are universal recipients because they can accept blood from anyone. Type A blood has A antigens on the blood cells and B antibodies in the plasma. The combination of B antibodies and B antigens will cause agglutination. There are also subsidiary blood proteins such as the rhesus factor (rh) that can be positive (present) or negative (absent). If only small amounts of blood are transfused, it is not a problem due to the dilution factor.

Immunochemistry is the chemistry of the **immune system**. Most of the chemicals involved in immune responses are proteins. Some chemicals inactivate invading proteins, others facilitate this response. The histocompatibility complex is a series of surface proteins on organs and tissues that elicit an immune response when placed in a genetically different individual.

See also Biochemistry; History of immunology; Immune stimulation, as a vaccine; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunization; Immunological analysis techniques; Laboratory techniques in immunology; Major histocompatibility complex (MHC)

IMMUNODEFICIENCY

The **immune system** is the body's main system to fight infections. Any defect in the immune system decreases a person's ability to fight infections. A person with an immunodeficiency disorder may get more frequent infections, heal more slowly, and have a higher incidence of some cancers.

The normal immune system involves a complex interaction of certain types of cells that can recognize and attack "foreign" invaders, such as **bacteria**, **viruses**, and **fungi**. It also plays a role in fighting cancer. The immune system has both innate and adaptive components. Innate **immunity** is made up of immune protections present at birth. Adaptive immunity develops the immune system to fight off specific invading organisms throughout life. Adaptive immunity is divided into two components: humoral immunity and cellular immunity.

The innate immune system is made up of the skin (which acts as a barrier to prevent organisms from entering the body), white blood cells called phagocytes, a system of proteins called the **complement** system, and chemicals called **interferons**. When phagocytes encounter an invading organism, they surround and engulf it to destroy it. The complement system also attacks bacteria. The elements in the complement system create a hole in the outer layer of the target cell, which leads to the death of the cell.

The adaptive component of the immune system is extremely complex, and is still not entirely understood. Basically, it has the ability to recognize an organism or tumor cell as not being a normal part of the body, and to develop a response to attempt to eliminate it.

The humoral response of adaptive immunity involves a type of cell called **B lymphocytes**. B lymphocytes manufacture proteins called antibodies (which are also called **immunoglobulins**). Antibodies attach themselves to the invading foreign substance. This allows the phagocytes to begin engulfing and destroying the organism. The action of antibodies also activates the complement system. The humoral response is particularly useful for attacking bacteria.

The cellular response of adaptive immunity is useful for attacking viruses, some **parasites**, and possibly cancer cells. The main type of cell in the cellular response is T lymphocytes. There are helper T lymphocytes and killer T lymphocytes. The helper T lymphocytes play a role in recognizing invading organisms, and they also help killer T lymphocytes to multiply. As the name suggests, killer T lymphocytes act to destroy the target organism.

Defects can occur in any component of the immune system or in more than one component (combined immunodeficiency). Different **immunodeficiency diseases** involve different components of the immune system. The defects can be inherited and/or present at birth (congenital), or acquired.

Congenital immunodeficiency is present at the time of birth, and is the result of genetic defects. Even though more than 70 different types of congenital immunodeficiency disorders have been identified, they rarely occur. Congenital immunodeficiencies may occur as a result of defects in B lymphocytes, T lymphocytes, or both. They can also occur in the innate immune system.

If there is an abnormality in either the development or function of B lymphocytes, the ability to make antibodies will be impaired. This allows the body to be susceptible to recurrent infections. Bruton's agammaglobulinemia, also known as X-linked agammaglobulinemia, is one of the most common congenital immunodeficiency disorders. The defect results in a decrease or absence of B lymphocytes, and therefore a decreased ability to make antibodies. People with this disorder are particularly susceptible to infections of the throat, skin, middle ear, and lungs. It is seen only in males because it is caused by a genetic defect on the X chromosome. Since males have only one X chromosome, they always have the defect if the **gene** is present. Females can have the defective gene, but since they have two X **chromosomes**, there will be a normal gene on the other X chromosome to counter it. Women may pass the defective gene on to their male children.

Another type of B lymphocyte deficiency involves a group of disorders called selective immunoglobulin deficiency syndromes. Immunoglobulin is another name for **antibody**,

and there are five different types of immunoglobulins (called IgA, IgG, IgM, IgD, and IgE). The most common type of immunoglobulin deficiency is selective IgA deficiency. The amounts of the other antibody types are normal. Some patients with selective IgA deficiency experience no symptoms, while others have occasional lung infections and diarrhea. In another immunoglobulin disorder, IgG and IgA antibodies are deficient and there is increased IgM. People with this disorder tend to get severe bacterial infections.

Common variable immunodeficiency is another type of B lymphocyte deficiency. In this disorder, the production of one or more of the immunoglobulin types is decreased and the antibody response to infections is impaired. It generally develops around the age of 10-20. The symptoms vary among affected people. Most people with this disorder have frequent infections, and some will also experience anemia and rheumatoid arthritis. Many people with common variable immunodeficiency develop cancer.

Severe defects in the ability of T lymphocytes to mature results in impaired immune responses to infections with viruses, fungi, and certain types of bacteria. These infections are usually severe and can be fatal.

DiGeorge syndrome is a T lymphocyte deficiency that starts during fetal development, but it isn't inherited. Children with DiGeorge syndrome either do not have a thymus or have an underdeveloped thymus. Since the thymus is a major organ that directs the production of **T-lymphocytes**, these patients have very low numbers of T-lymphocytes. They are susceptible to recurrent infections, and usually have physical abnormalities as well. For example, they may have low-set ears, a small receding jawbone, and wide-spaced eyes. In some cases, no treatment is required for DiGeorge syndrome because T lymphocyte production improves. Either an underdeveloped thymus begins to produce more T lymphocytes or organ sites other than the thymus compensate by producing more T lymphocytes.

Some types of immunodeficiency disorders affect both B lymphocytes and T lymphocytes. For example, **severe combined immunodeficiency** disease (**SCID**) is caused by the defective development or function of these two types of lymphocytes. It results in impaired humoral and cellular immune responses. SCID is usually recognized during the first year of life. It tends to cause a fungal infection of the mouth (**thrush**), diarrhea, failure to thrive, and serious infections. If not treated with a bone marrow transplant, a person with SCID will generally die from infections before age two.

Disorders of innate immunity affect phagocytes or the complement system. These disorders also result in recurrent infections.

Acquired immunodeficiency is more common than congenital immunodeficiency. It is the result of an infectious process or other disease. For example, the **Human Immunodeficiency Virus** (**HIV**) is the virus that causes acquired immunodeficiency syndrome (**AIDS**). However, this is not the most common cause of acquired immunodeficiency. Acquired immunodeficiency often occurs as a complication of other conditions and diseases. For example, the most common causes of acquired immunodeficiency are malnutrition, some types of cancer, and infections. People who weigh less than 70% of the average weight of persons of the same age and gender are considered to be malnourished. Examples of types of infections that can lead to immunodeficiency are chickenpox, cytomegalovirus, German **measles**, measles, **tuberculosis**, infectious **mononucleosis** (**Epstein-Barr virus**), chronic **hepatitis**, lupus, and bacterial and fungal infections.

Sometimes, acquired immunodeficiency is brought on by drugs used to treat another condition. For example, patients who have an organ transplant are given drugs to suppress the immune system so the body will not reject the organ. Also, some **chemotherapy** drugs, which are given to treat cancer, have the side effect of killing cells of the immune system. During the period of time that these drugs are being taken, the risk of infection increases. It usually returns to normal after the person stops taking the drugs.

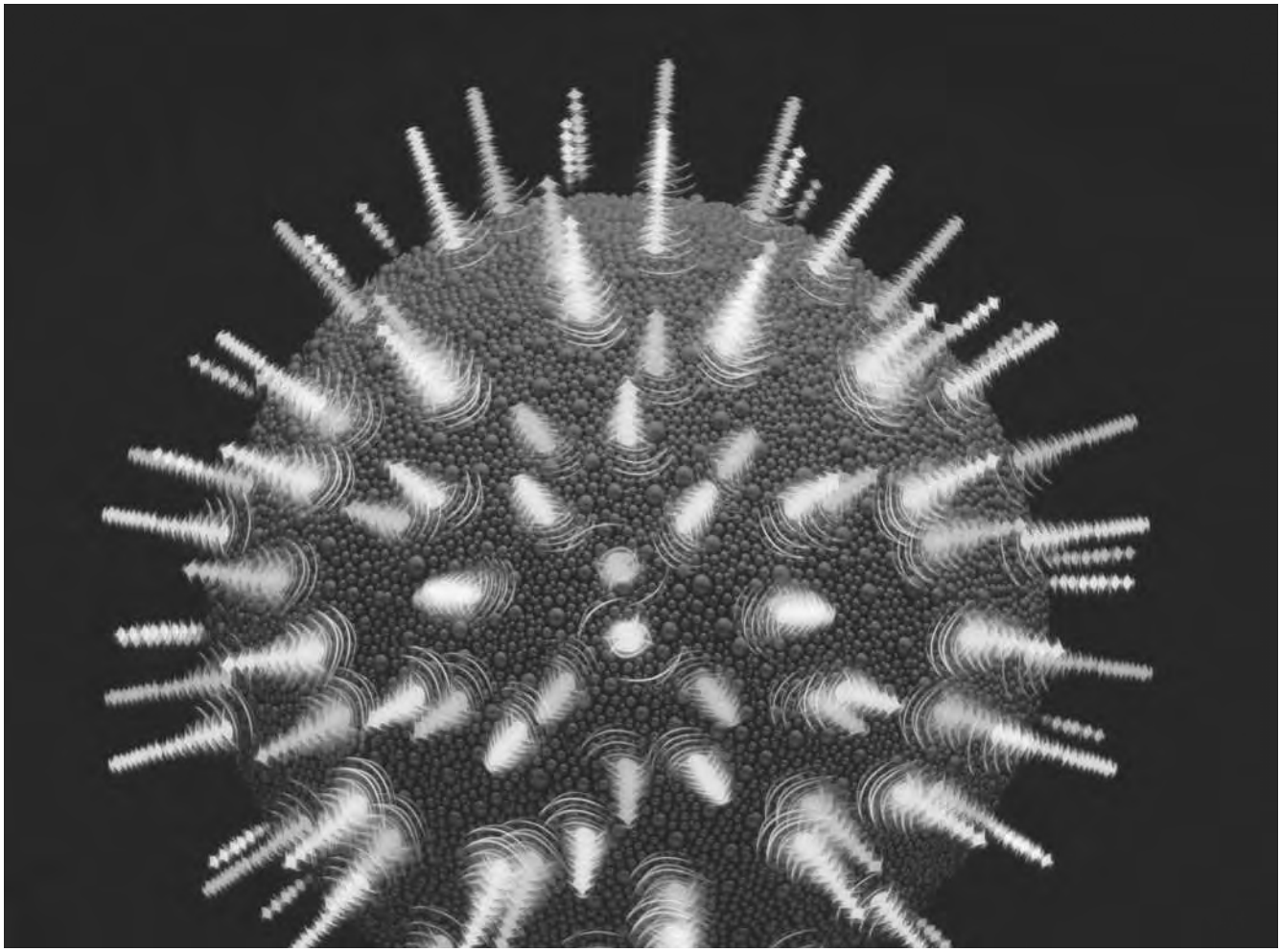
Congenital immunodeficiency is caused by genetic defects, and they generally occur while the fetus is developing in the womb. These defects affect the development and/or function of one or more of the components of the immune system. Acquired immunodeficiency is the result of a disease process, and it occurs later in life. The causes, as described above, can be diseases, infections, or the side effects of drugs given to treat other conditions.

People with an immunodeficiency disorder tend to become infected by organisms that don't usually cause disease in healthy persons. The major symptoms of most immunodeficiency disorders are repeated infections that heal slowly. These chronic infections cause symptoms that persist for long periods of time.

Laboratory tests are used to determine the exact nature of the immunodeficiency. Most tests are performed on blood samples. Blood contains antibodies, lymphocytes, phagocytes, and complement components—all of the major immune components that might cause immunodeficiency. A blood cell count will determine if the number of phagocytic cells or lymphocytes is below normal. Lower than normal counts of either of these two cell types correlates with immunodeficiencies. The blood cells are also checked for their appearance. Sometimes a person may have normal cell counts, but the cells are structurally defective. If the lymphocyte cell count is low, further testing is usually done to determine whether any particular type of lymphocyte is lower than normal. A lymphocyte proliferation test is done to determine if the lymphocytes can respond to stimuli. The failure to respond to stimulants correlates with immunodeficiency. Antibody levels can be measured by a process called **electrophoresis**. Complement levels can be determined by immunodiagnostic tests.

There is no cure for immunodeficiency disorders. Therapy is aimed at controlling infections and, for some disorders, replacing defective or absent components.

In most cases, immunodeficiency caused by malnutrition is reversible. The health of the immune system is directly linked to the nutritional health of the patient. Among the essential nutrients required by the immune system are proteins, vitamins, iron, and zinc. For people being treated for cancer, periodic relief from chemotherapy drugs can restore the function of the immune system.



Scanning electron microscope image of the Human Immunodeficiency Virus (HIV) on a hemocyte.

In general, people with immunodeficiency disorders should maintain a healthy diet. This is because malnutrition can aggravate immunodeficiencies. They should also avoid being near people who have colds or are sick because they can easily acquire new infections. For the same reason, they should practice good personal **hygiene**, especially dental care. People with immunodeficiency disorders should also avoid eating undercooked food because it might contain bacteria that could cause infection. This food would not cause infection in normal persons, but in someone with an immunodeficiency, food is a potential source of infectious organisms. People with immunodeficiency should be given **antibiotics** at the first indication of an infection.

There is no way to prevent a congenital immunodeficiency disorder. However, someone with a congenital immunodeficiency disorder might want to consider getting genetic counseling before having children to find out if there is a chance they will pass the defect on to their children.

Some of the infections associated with acquired immunodeficiency can be prevented or treated before they cause problems. For example, there are effective treatments for tuberculosis and most bacterial and fungal infections. HIV infection can be prevented by practicing "safe sex" and not using illegal intravenous drugs. These are the primary routes of transmitting the virus. For people who don't know the HIV status of the person with whom they are having sex, safe sex involves using a condom.

See also AIDS, recent advances in research and treatment; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunodeficiency disease syndromes; Immunodeficiency diseases; Infection and resistance

IMMUNODEFICIENCY DISEASE SYNDROMES

An effective **immune system** requires that any antigens that are not native to the body be quickly recognized and destroyed, and that none of the antigens native to the body be identified as

foreign. Excesses in the latter constitute the autoimmune diseases. Deficiencies in the body's ability to recognize antigens as foreign or a diminished capacity to respond to recognized antigens constitute the **immunodeficiency** syndromes.

There are many causes associated with immunodeficiencies. Primary immunodeficiencies are inherited conditions in which specific genes or **gene** families are corrupted by **mutations** or chromosome deletions. These syndromes are discussed elsewhere in this volume. Secondary immunodeficiencies are acquired conditions that may result from infections, cancers, aging, exposure to drugs, chemicals or radiation, or a variety of other disease processes.

Bacteria, viral, **fungi**, **protozoa**, and even parasitic infections can result in specific deficiencies of **B cells**, **T cells**, macrophages, and granulocytes. The best characterized of the infectious diseases is the acquired immunodeficiency syndrome (**AIDS**).

Infection by two **viruses**, HIV-1 and HIV-2, is associated with a wide range of responses in different people from essentially asymptomatic to a full-blown AIDS in which cell-mediated **immunity** is seriously compromised. HIV-1 and HIV-2 are **retroviruses** that attack humans and compromise cellular function. In contrast, the human T cell lymphotrophic viruses (**HTLV**) tend to provoke lymphoid neoplasms and neurologic disease. AIDS is most often associated with HIV-1 infection. The chance of developing AIDS following infection with HIV-1 is approximately one to two percent per year initially, and increases to around five percent per year after the fifth year of infection. Roughly, half of those infected with the virus will develop AIDS within ten years. In between those who are asymptomatic, and those with AIDS who are symptomatic with conditions associated with AIDS.

In AIDS, cellular immunity mechanisms are disrupted. Some immunologic cells are reduced in number and others, such as natural killer cells, have reduced activity despite their normal numbers. **HIV** infects primarily T helper lymphocyte cells and a variety of cells outside of the lymphoid system such as macrophages, endothelial, and epithelial cells. Because the T helper cells normally express a surface glycoprotein called CD4, counts of CD4 cells are helpful in predicting immunologic depression in HIV-infected individuals. The amount of viral **RNA** in circulation is also a helpful predictor of immunologic compromise. In addition to cell-mediated immunity, **antibody** responses (humoral immunity) are also muted in individuals with AIDS.

Initially, there is a period of several weeks to months where the host remains HIV antibody negative and viral replication occurs rapidly. Some subjects develop an acute response that appears like the flu or **mononucleosis**. Symptoms typically include fever, malaise, joint pain, and swollen lymph nodes. As the initial symptoms dissipate, patients enter an antibody positive phase without symptoms associated with AIDS. A variety of relatively mild symptoms like **thrush**, diarrhea, fever, or other viral infections may manifest along with a wide array of partial anemias. Nerve function can become compromised resulting in weakness, pain, or sensory loss. Eventually, life threatening opportunistic infections resulting from decreased immunologic function occur and may be accompanied by wasting, dementia, **meningitis**, and encephalitis. Drug therapy in the form of antiretroviral agents is directed toward inhibition of proteases and reverse transcriptase **enzymes** which are critical for replication of the viruses.

Although not nearly as well known as AIDS, there are a variety of other acquired immunodeficiencies. Infections other than HIV can significantly alter the numbers and functions of other cells within the immune system. While individually these various infections may appear to be relatively uncommon, depression in the numbers of platelets, T cells, B cells, natural killer (NK) cells, and granulocytes can lead to immunologic dysfunction. The manifestations of these various conditions will depend on the specific cell population that is involved and its normal function within the immune system. B cell deficiencies tend to result in an increased susceptibility to bacterial infections. Decreased natural killer cell activity can result in the survival of tumor cells which would otherwise be destroyed by the immune system.

Chemical and physical agents (such as radiation) also can potentially depress various fractions of cells within the immune system, and like the immunodeficiencies caused by infectious agents, the manifestations of these agents will differ depending on the cells which are influenced. Cancer chemotherapeutic agents are often immunosuppressive. Likewise, immune function often declines with age. T cell populations (including the T helper cells) decline as the thymus gland activity decreases. Frequently, B cell populations proliferate at an accelerated rate in older people. Over production of cells within the immune system such as leukemias, lymphomas, and related disorders also may disturb immune function by radically altering the distribution of white cells. A number of other diverse disease processes can alter or compromise immune function. These include diabetes, liver disease, kidney disease, sickle cell anemia, Down syndrome, and many of the autoimmune diseases.

See also AIDS, recent advances in research and treatment; Autoimmunity and autoimmune diseases; Immunodeficiency diseases, genetic causes

IMMUNODEFICIENCY DISEASES, GENETIC CAUSES

The complex workings of the **immune system** requires the cooperation of various organs, tissues, cells and proteins and thus, it can be compromised in a number of different ways. People who have normal immune function at birth who later acquire some form of **immunodeficiency** are said to have secondary or acquired immunodeficiency diseases. Examples would include **AIDS**, age-related immune depression, and other immune deficiencies caused by infections, drug reactions, radiation sickness, or cancer. Individuals who are born with an intrinsically reduced capacity for immunologic activity usually have some genetic alteration present at birth. There are varieties of different genes involved, and they render people susceptible to infection by an assortment of dif-

ferent germs. Some of these diseases are relatively mild with onset in adolescence or adulthood. Others are severely debilitating and severely compromise daily activity. Clinically significant primary immunodeficiencies are relatively rare with 1 in 5,000 to 1 in 10,000 people in developed countries afflicted.

The most common form of primary immunodeficiency, selective IgA deficiency, is a very mild deficiency and may affect as many as 1 in every 300 persons, most of whom will never realize they have an immunodeficiency at all. B-cells are lymphocytes that produce antibodies and this component of the immune system is often called humoral **immunity**. Defects in humoral immunity predispose the body to viral infections. T-cells are lymphocytes that are processed in the thymus gland. Granulocytes are cells which consume an destroy **bacteria**.

There are now thought to be around 70 different primary immunodeficiency diseases. Of the more common forms, the vast majority of these conditions are recessive. This means that a single working copy of the **gene** is generally sufficient to permit normal immune functioning. Some of the genes are found on the X chromosome. Since males receive only a single X chromosome, recessive **mutations** of these genes will result in disease. Females have two copies of the X chromosome, and so rarely will express X-linked recessive diseases.

The most widely known of the primary immunodeficiencies is severe combined immune deficiency (**SCID**) and it conjures pictures of a child who must live his life encased in a plastic bubble to keep out germs. SCID is manifest in early childhood as a severe combined T cell and B cell deficiency, and can be caused by a number of different gene mutations. The most common form is X-linked, and so primarily affects boys. It can also be caused by an enzyme called adenosine deaminase. When ADA is deficient, toxic chemicals kill off the lymphocytes. Until recently, SCID was uniformly lethal. In recent years, the elucidation of the genes responsible has made possible interventions based on gene therapy. SCID often presents in early childhood as persistent diaper rash or **thrush**. **Pneumonia**, **meningitis**, blood poisoning, and many common viral infections are serious threats to children born with SCID. Diagnosis demands immediate medical attention and bone marrow transplants are a common form of treatment for SCID. Children with ADA deficiency may be treated with ADA infusions to correct the enzyme deficiency. Partial combined immune deficiencies are milder conditions in which cellular and humoral immunity are both compromised but not completely shut down. These are generally accompanied by other physical symptoms and so constitute syndromes. Wiskott-Aldrich syndrome, for example, is an X-linked partial combined syndrome in which the repeated infections are combined with eczema and a tendency toward bleeding. Another combined B and T cell deficiency is ataxia telangiectasia (AT). In AT, the combined B and T cell deficiency causes repeated respiratory infections, and is accompanied by a jerky movement disorder and dilated blood vessels in the eyes and skin. The thymus gland where T-cells are processed is underdeveloped.

Deficiency of the B cell population results in decreased **antibody** production and thus, an increased risk of viral or **bacterial infection**. X-linked agammaglobulinemia (XLA) is a condition in which boys (because it is X-linked) produce little to no antibodies due to an absence of **B cells** and plasma cells in circulation. As these children grow, they deplete the antibodies transmitted through the mother, and they become susceptible to repeated infections. Common variable immunodeficiency (CVID) is a group of disorders in which the number of B cells is normal, but the levels of antibody production are reduced.

DiGeorge anomaly is an example of a T cell deficiency produced by an underdeveloped thymus gland. Children with DiGeorge anomaly often have characteristic facial features, developmental delays, and certain kinds of heart defects usually stemming from small deletions on chromosome 22 (or more rarely, chromosome 10). In rare cases, there is an autosomal dominant gene mutation rather than a chromosome deletion.

Phagocytosis, the ability of the granulocytes to ingest and destroy bacteria, can also be the chief problem. One example of this is chronic granulomatous disease (CGD). There are four known genes that cause CGD; all are recessive. One is on the X chromosome, and the other three are on autosomes. These children do well until around age three when they begin to have problems with staphylococcal infections and infections with **fungi** which are generally benign in other people. Their granulosa cells may aggregate in tissues forming tumor like masses. Similarly, leukocyte adhesion defect (LAD) is a condition in which granulocytes fail to work because they are unable to migrate to the site of infections. In Chediak-Higashi syndrome (CHS), not only granulocytes, but also melanocytes and platelets are diminished. CHS is generally fatal in adolescence unless treated by bone marrow transplantation.

One other class of primary immunodeficiencies, the **complement** system defects, result from the body's inability to recognize and/or destroy germs that have been bound by antibodies. Complement fixation is a complex multi step process, and thus a number of different gene mutations can potentially corrupt the normal pathway. Complement system defects are rare and often not expressed until later in life.

The prospect of the development of effective and safe gene therapies holds hope for the primary immunodeficiency diseases. As these genes and their genetic pathways are more fully understood, interventions which replace the missing gene product will likely provide effective treatments.

See also Immunity, cell mediated; Immunity, humoral regulation; Microbial genetics; Microbiology, clinical

IMMUNOELECTRON MICROSCOPY, THEORY, TECHNIQUES AND USES • *see*

ELECTRON MICROSCOPIC EXAMINATION OF MICROORGANISMS

IMMUNOELECTROPHORESIS

Immunoelectrophoresis is a technique that separates proteins on the basis of both their net charge (and so their movement in an electric field) and on the response of the **immune system** to the proteins. The technique is widely used in both clinical and research laboratories as a diagnostic tool to probe the protein composition of serum.

Petr Nikolaevich Grabar, a French immunologist, devised the technique in the 1950s. In essence, immunoelectrophoresis separates the various proteins in a sample in an electric field and then probes the separated proteins using the desired **antiserum**.

The most widely used version of the technique employs an apparatus, which consists basically of a **microscope** slide-sized plate. The plate is the support for a gel that is poured over top and allowed to congeal. The construction of the gel can vary, depending on the separation to be performed. **Agar**, such as that used in microbiological growth media, and another material called agarose can be used. Another popular choice is a linked network of a chemical known as acrylamide. The linked up acrylamide chains form what is designated as polyacrylamide.

The different types of gel networks can be most productively envisioned as a three-dimensional overlay of the crossed linked chains. The effect is to produce snaking tunnels through the matrix of various diameters. These diameters, which are also referred to as pore sizes, can be changed to a certain extent by varying the concentrations of some of the ingredients of the gel suspension. Depending on the size and the shape of the protein, movement through this matrix will be relatively slow or fast. As well, depending on the net charge a protein molecule has, the protein will migrate towards the positively charged electrode or the negatively charged electrode when the electric current is passed through the gel matrix. Thus, the various species of protein will separate from each other along the length of the gel.

In some configurations of the immunoelectrophoretic set-up, the samples that contain the proteins to be analyzed are added to holes on either side of the gel plate. For example, one sample could contain serum from a health individual and another sample could contain serum from someone with an infection. The middle portion of the plate contains a trough, into which a single purified species of **antibody** or known mixture of antibodies is added. The antibody molecules diffuse outward from the trough solution into the gel. Where an antibody encounters a corresponding **antigen**, a reaction causes the formation of a visual precipitate. Typically, the precipitation occurs in arc around the antigen-containing sample. In the example, the pattern of precipitation can reveal antigenic differences between the normal serum and the serum from a infected person.

This type of immunoelectrophoresis provides a qualitative ("yes or no") answer with respect to the presence or absence of proteins, and can be semi-quantitative. The shape of the arc of precipitation is also important. An irregularly shaped arc can be indicative of an abnormal protein or the presence of more than one antigenically similar protein.

Immunoelectrophoresis can also be used to detect a particular antigenic site following the transfer of the proteins from a gel to a special support, such as nitrocellulose. Addition of the antibody followed by a chemical to which bound antibody reacts produces a darkening on the support wherever antibody has bound to antigen. One version of this technique is termed Western Blotting. An advantage of this technique is that, by running two gels and using just one gel for the transfer of proteins to the nitrocellulose, the immune detection of a protein can be performed without affecting the protein residing in the other gel.

Another application of immunoelectrophoresis is known as capillary immunoelectrophoresis. In this application, a sample can be simultaneously drawn up into many capillary tubes. The very small diameter of the tubes means that little sample is required to fill a tube. Thus, a sample can be subdivided into very many sub volumes. Each volume can be tested against a different antibody preparation. Often, the reaction between antigen and antibody can be followed by the use of compounds that fluoresces when exposed to laser light of a specific wavelength. Capillary immunoelectrophoresis is proving to be useful in the study of Bovine Spongiform Encephalopathy in cattle, where sample sizes can be very small.

In the clinical laboratory setting, immunoelectophoresis is used to examine alterations in the content of serum, especially changes concerned with **immunoglobulins**. Change in the immunoglobulin profile can be the result of immunodeficiencies, chronic bacterial or viral infections, and infections of a fetus. The immunoglobulin most commonly assayed for are IgM, IgG, and IgA. Some of the fluids that can be examined using immunoelectrophoresis include urine, cerebrospinal fluid and serum. When concerned with immunoglobulins, the technique can also be called gamma globulin **electrophoresis** or immunoglobulin electrophoresis.

See also Antibody-antigen, biochemical and molecular reactions; Immunological analysis techniques

IMMUNOFLUORESCENCE

Immunofluorescence refers to the combination of an **antibody** and a compound that will fluoresce when illuminated by light of a specific wavelength. The duo is also referred to as a fluorescently labeled antibody. Such an antibody can be used to visually determine the location of a target **antigen** in biological samples, typically by microscopic observation.

The fluorescent compound that is attached to an antibody is able to absorb light of a certain wavelength, the particular wavelength being dependent on the molecular construction of the compound. The absorption of the light confers additional energy to the compound. The energy must be relieved. This is accomplished by the emission of light, at a higher wavelength (and so a different color) than the absorbed radiation. It is this release of radiant energy that is the underpinning for immunofluorescence.

Immunofluorescence microscopy can revel much detail about the processes inside cells. In a light microscopic application of the technique, sections of sample are exposed to the fluorescently labeled antibody. The large wavelength of visible light, relative to other forms of illumination such as laser light, does not allow details to be revealed at the molecular level. Still, details of the trafficking of a protein from the site of its manufacture to the surface of a cell, for example, is possible, by the application of different antibodies. The antibodies can be labeled with the same fluorescent compound but are applied at different times. An example of the power of this type of approach is the information that has been obtained concerning the pathway that the **yeast** known as *Saccharomyces cervisiae* uses to shuttle proteins out of the cell.

Resolution of details to the molecular level has been made possible during the 1990s with the advent of the technique of confocal laser microscopy. This technique employs a laser to sequentially scan samples at selected depths through the sample. These so-called optical sections can be obtained using laser illumination at several different wavelengths simultaneously. Thus, the presence of different antibodies that are labeled with fluorescent compounds that fluoresce at the different wavelengths can produce an image of the location of two antigens in the same sample at the same time.

The use of immunofluorescent compounds in combination with confocal microscopy has allowed the fluorescent probing of samples which do not need to be chemically preserved (or "fixed") prior to examination. The thin sections of sample that are examined in light microscopy often require such chemical fixation. While the fixation regimens have been designed to avoid change of the sample's internal structure, especially the chemistry and three-dimensional structure of the site of the antigen to which the antibody will bind, the avoidance of any form of chemical modification is preferred.

There are a multitude of fluorescent compounds available. Collectively these compounds are referred to as fluorochromes. A well-known example in biological and microbiological studies is the green fluorescent protein. This molecule is ring-like in structure. It fluoresces green when exposed to light in the ultraviolet or blue wavelengths. Other compounds such as fluorescein, rhodamine, phycoerythrin, and Texas Red, fluoresce at different wavelength and can produce different colors.

Immunofluorescence can be accomplished in a one-step or two-step reaction. In the first option, the fluorescently labeled antibody directly binds to the target antigen molecule. In the second option the target antigen molecule binds a so-called secondary antibody. Then, other antigenic sites in the sample that might also bind the fluorescent antibody are "blocked" by the addition of a molecule that more globally binds to antigenic sites. The secondary antibody then can itself be the target to which the fluorescently labeled antibody binds.

The use of antibodies to antigen that are critical to disease processes in **microorganisms** allow immunofluorescence to act as a detection and screening tool in the monitoring of a variety of materials. Foe example, research to adapt immunofluorescence to food monitoring is an active field. In the present, immunofluorescence provides the means by which organisms can be sorted using the technique of flow cytometry. As individual **bacteria**, for example, pass by a detector, the presence of fluorescence will register and cause the bacterium to be shuttled to a special collection reservoir. Thus, bacteria with a certain surface factor can be separated from the other bacteria in the population that do not possess the factor

See also Fluorescent dyes; Microscopy

IMMUNOGENETICS

Immunogenetics is the study of the mechanisms of autoimmune diseases, tolerance in organ transplantation, and **immunity** to infectious diseases—with a special emphasis on the role of the genetic make-up of an organism in these processes. The **immune system** evolved essentially to protect vertebrates from a myriad species of potentially harmful infectious agents such as **bacteria**, virus, **fungi** and various eukaryotic **parasites**. However, the growing understanding of the immune system has influenced a variety of different biomedical disciplines, and is playing an increasingly important role in the study and treatment of many human diseases such as cancer and autoimmune conditions.

There are two broad types of immune systems. The innate immune system of defense depends on invariant receptors that recognize common features of pathogens, but are not varied enough to recognize all types of pathogens, or specific enough to act effectively against re-infection by the same pathogen. Although effective, this system lacks both specificity and the ability to acquire better receptors to deal with the same infectious challenge in the future, a phenomenon called immunological memory. These two properties, specificity and memory, are the main characteristics of the second type of immune system, known as the specific or adaptive immune system, which is based on **antigen** specific receptors. Besides these two families of different receptors that help in immune recognition of foreign infectious agents, both the innate and the adaptive immune systems rely on soluble mediators like the different **cytokines** and kemokines that allow the different cells involved in an immune response to communicate with each other. The major focus of immunogeneticists is the identification, characterization, and sequencing of genes coding for the multiple receptors and mediators of immune responses.

Historically, the launch of immunogenetics could be traced back to the demonstration of Mendelian inheritance of the human ABO blood groups in 1910. The importance of this group of molecules is still highlighted by their important in blood transfusion and organ transplantation protocols. Major developments that contributed to the emergence of immunogenetics as an independent discipline in **immunology** were the rediscovery of allograft reactions during the Second World War and the formulation of an immunological theory of allograft reaction as well as the formulation of the clonal **selection** hypothesis by Burnett in 1959. This theory proposed that clones of immunocompetent cells with unique receptors exist prior to exposure to antigens, and only cells with specific receptors are selected by antigen for subsequent activation.

The molecular understanding of how the diverse repertoire of these receptors is generated came with the discovery of somatic **recombination** of receptor genes, which is the paradigm for studying **gene** rearrangement during cell maturation.

The most important influence on the development of immunogenetics is, however, the studies of a gene family known as the MCH, or **major histocompatibility complex**. These highly polymorphic genes, first studied as white-cell antigens of the blood and therefore named human leukocyte antigens (**HLA**), influence both donor choice in organ transplantation and the susceptibility of an organism to chronic diseases. The **MHC** is also linked with most of all the important autoimmune diseases such as rheumatoid arthritis and diabetes.

The discovery in 1972 that these MHC molecules are intimately associated with the specific immune response to **viruses** led to an explosion in immunogenetic studies of these molecules. This has led to the construction of very detailed genetic and physical maps of this complex and ultimately to its complete sequence in an early stage of the human genome-sequencing project.

Other clusters of immune recognition molecules that are well established at the center of the immunogenetics discipline are the large arrays of rearranging gene segments that determine B-cell **immunoglobulins** and T-cell receptors. Immunoglobulins, which mediate the humoral immune response of the adaptive immune system, are the antibodies that circulate in the bloodstream and diffuse in other body fluids, where they bind specifically to the foreign antigen that induced them. This interaction with the antigen most often leads to its clearance. T cell receptors, which are involved in the cell-mediated immune response of the adaptive immune system, are the principle partners of the MHC molecules in mounting a specific immune response. An antigen that is taken up by specialized cells called antigen presenting cells is usually presented on the surface of this cell in complex with either MHC class I or class II to **T cells** that use specific receptors to recognize and react to the infectious agent. The reacting T cells can kill the host cells that bear the foreign antigen or secrete mediators (cytokines and lynphokines) that activate professional phagocytic cells of the immune system that eliminate the antigen. It is believed that during disease **epidemics**, some forms of class I and class II MHC molecules stimulate T-cell responses that better favor survival. Which MHC molecule is more favorable depends on the infectious agents encountered. Consequently, human populations that were geographically separated and have different disease histories differ in the sequences and frequencies of the HLA class I and class II alleles.

Other immune recognition molecules that were studied in great details in immunogenetics are two families of genes that encode receptors on the surface for natural killer (NK) cells. These large lymphocytes participate in the innate immune system and provide early defense from a pathogens attack, a response that distinguish them from B and T cells which become useful after days of infection. Some NK-cell receptors bind polymorphic determinants of MHC class I molecules and appear to be modulated by the effects that infectious agents have upon the conformation of these determinants.

One of the most important applications of immunogenetics in clinical medicine is HLA-typing in order to help match organ donors and recipients during transplantation surgery. Transplantation is a procedure in which an organ or tissue that is damaged and is no longer functioning is replaced with one obtained from another person. Because HLA antigens can be recognized as foreign by another person's immune system, surgeons and physicians try to match as many of the HLA antigens as possible, between the donated organ and the recipient. In order to do this, the HLA type of every potential organ recipient is determined. When a potential organ donor becomes available, the donor's HLA type is determined as well to make absolutely sure that the donor organ is suitable for the recipient.

See also Autoimmunity and autoimmune diseases; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunologic therapies; Immunosuppressant drugs; *In vitro* and *in vivo* research; Laboratory techniques in immunology; Major histocompatibility complex (MHC); Medical training and careers in immunology; Molecular biology and molecular genetics; Mutations and mutagenesis; Oncogenetic research; Transplantation genetics and immunology; Viral genetics

IMMUNOGLOBULIN DEFICIENCY • *see*

IMMUNODEFICIENCY DISEASE SYNDROMES

IMMUNOGLOBULINS AND IMMUNOGLOBULIN DEFICIENCY SYNDROMES

Immunoglobulins are proteins that are also called antibodies. The five different classes of immunoglobulins are formed in response to the presence of antigens. The specificity of an immunoglobulin for a particular **antibody** is exquisitely precise

The five classes of immunoglobulins are designated IgA, IgD, IgG, IgE, and IgM. These share a common structure. Two so-called heavy chains form a letter "Y" shape, with two light chains linked to each of the upper arms of the Y. The heavy chains are also known as *alpha, delta, gamma, epsilon,* or *mu*. The light chains are termed *lambda* or *kappa*.

The IgG class of immunoglobulin is the most common. IgG antibody is routinely produced in response to bacterial and viral infections and to the presence of toxins. IgG is found in many tissues and in the plasma that circulates throughout the body. IgM is the first antibody that is produced in an immune response. IgA is also produced early in a body's immune response, and is commonly found in saliva, tears, and other such secretions. The activity of IgD is still not clear. Finally, the IgE immunoglobulin is found in respiratory secretions.

The different classes of immunoglobulins additionally display differences in the sequence of amino acids comprising certain regions within the immunoglobulin molecule. For example, differences in the antigen-binding region, the variable region, accounts for the different **antigen** binding specificities

of the various immunoglobulins. Differences in their structure outside of the antigen binding region, in an area known as the constant region, accounts for differences in the immunoglobulin in other functions. These other functions are termed effector functions, and include features such as the recognition and binding to regions on other cells, and the stimulation of activity of an immune molecule known as **complement**.

The vast diversity of immunoglobulin specificity is due to the tremendous number of variations that are possible in the variable region of an immunoglobulin. A certain immune cell known as a B cell produces each particular immunoglobulin. Thus, at any particular moment in time, there are a myriad of **B cells** actively producing a myriad of different immunoglobulins, in response to antigenic exposure.

Immunoglobulins can exist in two forms. They can be fixed to the surface of the B cells that have produced them. Or they can float freely in body fluids, essentially patrolling until a recognizable antigen is encountered. The protection of the body from invading antigens depends on the production of immunoglobulins of the required type and in sufficient quantity.

Conditions where an individual has a reduced number of immunoglobulins or none at all of a certain type is known as an immunoglobulin deficiency syndrome. Such syndromes are typically the result of damage to B cells.

People with immunoglobulin deficiencies are prone to more frequent illness than those people whose immune systems are fully functional. Often the illnesses are caused by **bacteria**, in particular bacteria that are able to form a capsule surrounding them. A capsule is not easily recognized by even an optimally performing **immune system**. As well, immunoglobulin deficiency can render a person more susceptible to some viral infections, in particular those caused by echovirus, enterovirus, and **hepatitis** B.

Immunoglobulin deficiencies can take the form of a primary disorder or a secondary disorder. A secondary deficiency results from some other ongoing malady or treatment. For example, **chemotherapy** for a cancerous illness can compromise the immune system, leading to an **immunodeficiency**. Once the treatment is stopped the immunodeficiency can be reversed. A primary immunodeficiency is not the result of an illness or medical treatment. Rather, it is the direct result of a genetic disorder or a defect to B cells or other immune cells.

X-linked agammaglobulinemia results in an inability of B cells to mature. This results in the production of fewer B cells and in a lack of "memory" of an infection. Normally, the immune system is able to rapidly respond to antigen that has been encountered before, because of the "memory" of the B cells. Without this ability, repeated infections caused by the same agent can result.

Another genetically based immunoglobulin deficiency is known as selective IgA deficiency. Here, B cells fail to switch from producing IgM to produce IgA. The limited amount of IgA makes someone more prone to infections of mucosal cells. Examples of such infections include those in the nose, throat, lungs, and intestine.

Genetic abnormalities cause several other immunodeficiency syndromes. A missing stretch of information in the **gene** that codes for the heavy chain of IgG results in the production of an IgG that is structurally incomplete. The result is a loss of function of the IgG class of antibodies, as well as the IgA and IgE classes. On a subtler level, another genetic malfunction affects the four subclasses of antibodies within the IgG class. The function of some of the subclasses are affected more so than other subclasses. Finally, another genetic mutation destroys the ability of B cells to switch from making IgM to manufacture IgG. The lack of flexibility in the antibody capability of the immune system adversely affects the ability of the body to successfully fight infections.

Transient hypogammaglobulinemia is an immunodeficiency syndrome that is not based on a genetic aberration. Rather, the syndrome occurs in infants and is of short-term in duration. The **T cells** of the immune system do not function properly. Fewer than normal antibodies are produced, and those that are made are poor in their recognition of the antigenic target. However, as the immune system matures with age the proper function of the T cells is established. The cause of the hypogammaglobulinemia is not known.

Immunoglobulin deficiency syndromes are curable only by a bone marrow transplant, an option exercised in life-threatening situations. Normally, treatment rather than cure is the option. Prevention of infection, through the regular use of antimicrobial drugs and scrupulous oral health are important to maintain health in individuals with immunoglobulin deficiency syndromes.

See also Immunochemistry; Immunodeficiency disease syndromes; Immunodeficiency diseases; Immunodeficiency; Immunogenetics; Immunologic therapies; Immunological analysis techniques; Immunology; Immunosuppressant drugs

IMMUNOLOGIC THERAPIES

Immunologic therapy is defined as the use of medicines that act to enhance the body's immune response as a means of treating disease. The drugs can also aid in the recovery of the body from the harmful effects of immune-compromising treatments like **chemotherapy** and radiation.

Both microorganism-related infections and other maladies that are due to immune deficiency or cell growth defects are targets of immunologic therapy.

The emphasis in immunologic therapy is the application of synthetic compounds that mimic immune substances that are naturally produced in the body. For example, a compound called aldesleukin is an artificial form of interkeukin-2, a natural compound that assists white blood cells in recognizing and dealing with foreign material. Other examples are filgrastim and sargramostim, which are synthetic version of **colony** stimulating factors, which stimulate bone marrow to make the white blood cells, and epoetin, an artificial version of erythropoietin, which stimulates the marrow to produce red blood cells. Thrombopoietin encourages the manufacture of platelets, which are plate-shaped components of the blood that are vital in the clotting of blood. As a final example, synthetic forms of interferon are available and can be administered to

aid the natural forms of interferon in battling infections and even cancer.

Research has provided evidence that the infusion of specific **enzymes** can produce positive results with respect to some neurological disorders. While not strictly an immunologic therapy, the supplementation of the body's natural components is consistent with the aim of the immune approach.

The use of immunologic therapy is not without risk. Paradoxically, given their longer-term enhancement of the immune defenses, some of the administered drugs reduce the body's ability to fight off infection because of a short-term damping-down of some aspects of the **immune system**. As well, certain therapies carry a risk of reduced clotting of the blood and of seizures.

As with other therapies, the use of immunologic therapies is assessed in terms of the risks of the therapy versus the health outcome if therapy is not used. Typically, the immediate health threat to a patient outweighs the possible side effects from therapy. Immunologic therapies are always administered under a physician's care, almost always in a hospital setting. As well, frequent monitoring of the patients is done, both for the abatement of the malady and the development of adverse effects.

Immunologic therapy can provide continued treatment following chemotherapy or the use of radiation. The latter two treatments cannot be carried on indefinitely, due to toxic reactions in the body. Immunologic therapy provides another avenue of treatment. For example, some tumors that are resistant to chemical therapy are susceptible to immune attack. By enhancing the immune response, such tumors may be productively treated. Moreover, despite their side effects, immunologic therapies usually are less toxic than either chemotherapy or the use of radiation.

See also Immune system; Laboratory techniques in immunology

IMMUNOLOGICAL ANALYSIS TECHNIQUES

Immunological techniques are the wide varieties of methods and specialized experimental protocols devised by immunologists for inducing, measuring, and characterizing immune responses. They allow the immunologists to alter the **immune system** through cellular, molecular and genetic manipulation. These techniques are not restricted to the field of **immunology**, but are widely applied by basic scientists in many other biological disciplines and by clinicians in human and veterinary medicine.

Most immunological techniques available are focused on the study of the adaptive immune system. They classically involve the experimental induction of an immune response using methods based on **vaccination** protocols. During a typical experiment called **immunization**, immunologists inject a test **antigen** to an animal or human subject and monitor for the appearance of immune responses in the form of specific antibodies and effector **T cells**. Monitoring the **antibody** response usually involves the analysis of crude preparations of serum from the immunized subject. The analysis of the immune responses mediated by T cells are usually performed only on experimental animals and involves the preparation of these cells from blood or from the lymphoid organs, such as the spleen and the lymph nodes. Typically, any substance that has a distinctive structure or conformation that may be recognized by the immune system can serve as an antigen. A wide range of substances from simple chemicals like sugars, and small peptides to complex macromolecules and **viruses** can induce the immune system. Although the antigenic determinant of a test substance is usually a minor part of that substance called the epitope, a small antigen referred to as a hapten can rarely elicit an immune response on its own. It is not an immunogen and would therefore need to be covalently linked to a carrier in order to elicit an immune response. The induction of such a response to even large immunogenic antigen is not easy to achieve and the dose, the form and route of administration of that antigen can profoundly affect whether a response can occur. Especially the use of certain substances called adjuvants is necessary to alert the immune system and produce a strong immune response.

According to the clonal **selection** theory, antibodies produced in a typical immunization experiment are products of different clones of B-lymphocytes that are already committed to making antibodies to the corresponding antigen. These polyclonal antibodies are multi-subunit proteins that belong to the **immunoglobulins** family. They have a basic Y-shaped structure with two identical Fab domains, which form the arms and interact with the antigen, and one Fc domain that forms the stem and determines the isotype subclass of each antibody. There are five different isotype subclasses, IgM, Ig G, IgA, IgE, and IgD, which show different tissue distribution and half-life *in vivo*. They determine the biological function of the antibodies and appear during different stages of the immunization process. Knowledge about the biosynthesis and structure of these antibodies is important for their detection and use both as diagnostic and therapeutic tools.

Antibodies are highly specific for their corresponding antigen, and are able to detect one molecule of a protein antigen out of around a billion similar molecules. The amount and specificity of an antibody in a test serum can be measured by its direct binding to the antigen in assays usually referred to as primary interaction immunoassays. Commonly used direct assays are radioimmunoassay (RIA), enzyme-linked immunosorbent assay (**ELISA**), and immunoblotting techniques. In both ELISA and RIA, an enzyme or a radioisotope is covalently linked to the pure antigen or antibody. The unlabeled component, which most often is the antigen, is attached to the surface of a plastic well. The labeled antibody is allowed to bind to the unlabeled antigen. The plastic well is subsequently washed with plenty of **buffer** that will remove any excess non-bound antibody and prevent non-specific binding. Antibody binding is measured as the amount of radioactivity retained by the coated wells in radioimmunoassay or as fluorescence emitted by the product of an enzymatic reaction in the case of ELISA. Modifications of these assays known as competitive inhibition assays can be used that will allow quantifying the antigen (or antibody) in a mixture and determining the affinity of the antibody-antigen interaction by using math-

ematical models. Immunoblotting is usually performed in the form of Western blotting, which is reserved to the detection of proteins and involves an **electrophoresis** separation step followed by electroblotting of the separated proteins from the gel to a membrane and then probing with an antibody. Detection of the antigen protein antibody interaction is made in a similar way as in RIA or ELISA depending on whether a radiolabeled or enzyme-coupled antibody is used.

Antibodies can also be monitored through immunoassays that are based on the ability of antibodies to alter the physical state of their corresponding antigens and typically involve the creation of a precipitate in a solid or liquid medium. The hemmaglutination assay used to determine the ABO type of blood groups and match compatible donors and recipients for blood transfusion is based on this assay. Currently, the most common application of this immunoassay is in a procedure known as immunoprecipitation. This method allows antibodies to form complexes with their antigen in a complex mixture like the cytosol, the **nucleus** or membrane complexes of the cell. The antigen-antibody complex is precipitated either by inducing the formation of even larger complexes through the addition of excess amounts of anti-immunoglobulin antibodies or by the addition of agarose beads coupled to a special class of bacterial proteins that bind the Fc region of the antibody. The complex can also be precipitated by covalently linking the antibody to agarose beads forming a special affinity matrix. This procedure will also allow the purification of the antigen by immunoaffinity, a special form of affinity chromatography. Immunoprecipitation is a valuable technique that led to major discoveries in immunology an all disciplines of molecular and cellular biology. It allows the precipitation of the antigen in complex with other interacting proteins and reagents and therefore gives an idea on the function of the antigen.

The T cell immune response is detected by using monoclonal antibodies, a specific family of antibodies that recognize surface markers that are expressed by lymphocytes upon their activation. These monoclonal antibodies are highly specific, and are produced by special techniques from single clones of **B cells** and are therefore, homogenous groups of immunoglobulins with the same isotype and antigen binding affinity. These antibodies are used to identify characterize cells by flow cytometry (FACS), immunocytochemistry, **immunofluorescence** techniques. The difficulty to isolate antigen specific T cells is due to the fact that these T cells recognize the antigen in the context of a tri-molecular complex involving the T cell receptor and the **MHC** molecules on the surface of specialized cells called antigen-presenting cells. These interactions are subtle, have low affinity and are extremely complex to study. Novel and powerful techniques using tetramers of MHC molecules were developed in 1997 that are now used to identify and isolate antigen specific T cell clones. These tetramer-based assays are proving useful in separating very rare cells, and could be used in clinical medicine. In fact, virus and tumor specific T cells usually give a stronger response and are usually more effective in killing virus infected and tumor cells. Testing for the function of activated, antigen specific T cells known as effector T cells is routinely done *in vitro* by testing for cytokine production, cytotoxicity to other cells and proliferation in response to antigen stimulation. Local reactions in the skin of animals and humans provide information about T cell responses to an antigen, a procedure that is very used in testing for allergic reactions and the efficacy of vaccination procedures. Experimental manipulations of the immune system in vivo are performed to reveal the functions of each component of the immune system *in vivo*. **Mutations** through irradiation, or mutations produced by **gene** targeting (e.g., knock-out and knock-in techniques), as well as animal models produced by transgenic breeding, are proving helpful to researchers in evaluating this highly complex system.

See also Immune complex test; Immune stimulation, as a vaccine; Immune synapse; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunization; Immunochemistry; Immunodeficiency; Immunoelectrophoresis; Immunofluorescence; Immunogenetics; Immunologic therapies; Immunology; Immunomodulation; Immunosuppressant drugs; *In vitro* and *in vivo* research; Laboratory techniques in immunology

IMMUNOLOGICAL ASPECTS OF REPRODUCTION • *see* REPRODUCTIVE IMMUNOLOGY

IMMUNOLOGY

Immunology is the study of how the body responds to foreign substances and fights off infection and other disease. Immunologists study the molecules, cells, and organs of the human body that participate in this response.

The beginnings of our understanding of **immunity** date to 1798, when the English physician **Edward Jenner** (1749–1823) published a report that people could be protected from deadly **smallpox** by sticking them with a needle dipped in the material from a **cowpox** boil. The French biologist and chemist **Louis Pasteur** (1822–1895) theorized that such **immunization** protects people against disease by exposing them to a version of a microbe that is harmless but is enough like the disease-causing organism, or pathogen, that the **immune system** learns to fight it. Modern vaccines against diseases such as **measles**, polio, and chicken pox are based on this principle.

In the late nineteenth century, a scientific debate was waged between the German physician **Paul Ehrlich** (1854–1915) and the Russian zoologist **Élie Metchnikoff** (1845–1916). Ehrlich and his followers believed that proteins in the blood, called antibodies, eliminated pathogens by sticking to them; this phenomenon became known as humoral immunity. Metchnikoff and his students, on the other hand, noted that certain white blood cells could engulf and digest foreign materials: this cellular immunity, they claimed, was the real way the body fought infection.

Modern immunologists have shown that both the humoral and cellular responses play a role in fighting disease.

They have also identified many of the actors and processes that form the immune response.

The immune response recognizes and responds to pathogens via a network of cells that communicate with each other about what they have "seen" and whether it "belongs." These cells patrol throughout the body for infection, carried by both the blood stream and the lymph ducts, a series of vessels carrying a clear fluid rich in immune cells.

The **antigen** presenting cells are the first line of the body's defense, the scouts of the immune army. They engulf foreign material or **microorganisms** and digest them, displaying bits and pieces of the invaders—called antigens—for other immune cells to identify. These other immune cells, called T lymphocytes, can then begin the immune response that attacks the pathogen.

The body's other cells can also present antigens, although in a slightly different way. Cells always display antigens from their everyday proteins on their surface. When a cell is infected with a virus, or when it becomes cancerous, it will often make unusual proteins whose antigens can then be identified by any of a variety of cytotoxic T lymphocytes. These "killer cells" then destroy the infected or cancerous cell to protect the rest of the body. Other T lymphocytes generate chemical or other signals that encourage multiplication of other infection-fighting cells. Various types of T lymphocytes are a central part of the cellular immune response; they are also involved in the humoral response, encouraging **B lymphocytes** to turn into antibody-producing plasma cells.

The body cannot know in advance what a pathogen will look like and how to fight it, so it creates millions and millions of different lymphocytes that recognize random antigens. When, by chance, a B or T lymphocyte recognizes an antigen being displayed by an antigen presenting cell, the lymphocyte divides and produces many offspring that can also identify and attack this antigen. The way the immune system expands cells that by chance can attack an invading microbe is called clonal **selection**.

Some researchers believe that while some B and T lymphocytes recognize a pathogen and begin to mature and fight an infection, others stick around in the bloodstream for months or even years in a primed condition. Such memory cells may be the basis for the immunity noted by the ancient Chinese and by Thucydides. Other immunologists believe instead that trace amounts of a pathogen persist in the body, and their continued presence keeps the immune response strong over time.

Substances foreign to the body, such as disease-causing **bacteria**, **viruses**, and other infectious agents (known as antigens), are recognized by the body's immune system as invaders. The body's natural defenses against these infectious agents are antibodies—proteins that seek out the antigens and help destroy them. Antibodies have two very useful characteristics. First, they are extremely specific; that is, each **antibody** binds to and attacks one particular antigen. Second, some antibodies, once activated by the occurrence of a disease, continue to confer resistance against that disease; classic examples are the antibodies to the childhood diseases chickenpox and measles.

The second characteristic of antibodies makes it possible to develop vaccines. A **vaccine** is a preparation of killed or weakened bacteria or viruses that, when introduced into the body, stimulates the production of antibodies against the antigens it contains.

It is the first trait of antibodies, their specificity, that makes monoclonal antibody technology so valuable. Not only can antibodies be used therapeutically, to protect against disease; they can also help to diagnose a wide variety of illnesses, and can detect the presence of drugs, viral and bacterial products, and other unusual or abnormal substances in the blood.

Given such a diversity of uses for these disease-fighting substances, their production in pure quantities has long been the focus of scientific investigation. The conventional method was to inject a laboratory animal with an antigen and then, after antibodies had been formed, collect those antibodies from the blood serum (antibody-containing blood serum is called **antiserum**). There are two problems with this method: It yields antiserum that contains undesired substances, and it provides a very small amount of usable antibody.

Monoclonal antibody technology allows the production of large amounts of pure antibodies in the following way. Cells that produce antibodies naturally are obtained along with a class of cells that can grow continually in cell **culture**. The hybrid resulting from combining cells with the characteristic of "immortality" and those with the ability to produce the desired substance, creates, in effect, a factory to produce antibodies that work around the clock.

A myeloma is a tumor of the bone marrow that can be adapted to grow permanently in cell culture. Fusing myeloma cells with antibody-producing mammalian spleen cells, results in hybrid cells, or hybridomas, producing large amounts of monoclonal antibodies. This product of cell fusion combined the desired qualities of the two different types of cells, the ability to grow continually, and the ability to produce large amounts of pure antibody. Because selected hybrid cells produce only one specific antibody, they are more pure than the polyclonal antibodies produced by conventional techniques. They are potentially more effective than conventional drugs in fighting disease, because drugs attack not only the foreign substance but also the body's own cells as well, sometimes producing undesirable side effects such as nausea and allergic reactions. Monoclonal antibodies attack the target molecule and only the target molecule, with no or greatly diminished side effects.

While researchers have made great gains in understanding immunity, many big questions remain. Future research will need to identify how the immune response is coordinated. Other researchers are studying the immune systems of nonmammals, trying to learn how our immune response evolved. Insects, for instance, lack antibodies, and are protected only by cellular immunity and chemical defenses not known to be present in higher organisms.

Immunologists do not yet know the details behind allergy, where antigens like those from pollen, poison ivy, or certain kinds of food make the body start an uncomfortable, unnecessary, and occasionally life-threatening immune response. Likewise, no one knows exactly why the immune

system can suddenly attack the body's tissues—as in autoimmune diseases like rheumatoid arthritis, juvenile diabetes, systemic lupus erythematosus, or multiple sclerosis.

The hunt continues for new vaccines, especially against parasitic organisms like the **malaria** microbe that trick the immune system by changing their antigens. Some researchers are seeking ways to start an immune response that prevents or kills cancers. A big goal of immunologists is the search for a vaccine for **HIV**, the virus that causes **AIDS**. HIV knocks out the immune system—causing immunodeficiency—by infecting crucial T lymphocytes. Some immunologists have suggested that the chiefly humoral response raised by conventional vaccines may be unable to stop HIV from getting to lymphocytes, and that a new kind of vaccine that encourages a cellular response may be more effective.

Researchers have shown that transplant rejection is just another kind of immune response, with the immune system attacking antigens in the transplanted organ that are different from its own. Drugs that suppress the immune system are now used to prevent rejection, but they also make the patient vulnerable to infection. Immunologists are using their increased understanding of the immune system to develop more subtle ways of deceiving the immune system into accepting transplants.

See also AIDS, recent advances in research and treatment; Antibody, monoclonal; Biochemical analysis techniques; BSE, scrapie and CJD: recent advances in research; History of immunology; Immunochemistry; Immunodeficiency disease syndromes; Immunodeficiency diseases; Immunodeficiency; Immunogenetics; Immunological analysis techniques; Immunology, nutritional aspects; Immunosuppressant drugs; Infection and resistance; Laboratory techniques in immunology; Reproductive immunology; Transplantation genetics and immunology

IMMUNOLOGY, HISTORY OF • *see* HISTORY OF IMMUNOLOGY

IMMUNOLOGY, NUTRITIONAL ASPECTS

The role of nutrition is central to the development and modulation of the **immune system**. The importance of nutrition has been made clear by the burgeoning field of sports medicine. It appears the immune system is enhanced by moderate to severe exercise, although many components of the immune response exhibit adverse change for a period of from 3 to 72 hours after prolonged intense exertion. This "window of opportunity" for opportunistic bacterial and viral infections seems to be increased for "elite" athletes that are more prone to over-train. The elements of the immune response most affected by the strenuous activity that leads to the impairment of the immune system are lymphocyte concentrations, depressed natural killer activity, and elevated levels of IgA in the saliva.

The possible basis for this prolonged immunosuppression may include reduced plasma glutamine concentrations, altered plasma glucose levels, and proliferation of neutrophils and monocytes that increases prostaglandin concentrations. Exercise produces oxidative stress and so concomitantly, there are elevated free radical levels along with an attendant depletion of antioxidant levels. Therefore, antioxidants that help protect against oxidative stress are considered the most promising for further study, but those nutrients that heal the gut show potential also. These nutrients include Vitamin E, Vitamin C, zinc, and glutamine. Glutamine and nucleotides show a direct effect on lymphocyte proliferation. Free radicals and other reactive oxygen species that can damage cells as well as tissues are an integral part of the immune system, so the body has developed systems that protect from their damage. These products function by destroying invading organisms and damaged tissues, as well as enhance interleukin-I, Interleukin-8 and tumor necrosis factor concentrations as part of the inflammatory response. The purpose of supplementing the diet is to provide a balance to the immune system's prooxidant function. Carbohydrate supplementation has additionally shown impressive results. Increased plasma levels, a depressed cortisol and growth hormone response, fewer fluctuations in blood levels of immune competent cells, decreased granulocyte and monocyte **phagocytosis**, reduced oxidative stress and a diminished pro-inflammatory and anti-inflammatory cytokine response are all associated with an increase in complex carbohydrate consumption.

Besides exercise-associated immune suppression, malnutrition plays a pivotal role in modulating the immune response. Nowhere is this more important then during pregnancy and gestation. Besides genetics, no other factor is more important for the developing immune system then optimal nutrition. The immune response of low-birth-weight babies is compromised as well as those of children born to mothers without adequate nutrition. Especially important is the role of Vitamin E and selenium in preventing immune impairment. Animal studies showed that progeny of Vitamin E and selenium-deficient mothers never adequately developed immune competent cell lines.

Because nutrition plays such a vital role in the immune response, a special branch of **immunology** is developing called immunonutrition. These scientists are particularly interested in the interaction of genetics and nutrition. Preliminary work suggests that individual genotypes vary in their response to healing, infection, and dietary supplementation.

See also Immunogenetics; Infection and resistance; Metabolism

IMMUNOMODULATION

From a therapeutic point of view, immunomodulation refers to any process in which an immune response is altered to a desired level. **Microorganisms** are also capable of modulating the response of the **immune system** to their presence, in order

to establish or consolidate an infection. Thus, immunomodulation can be beneficial or detrimental to a host.

Many providers of nutritional supplements claim that a product enhances certain aspects of the immune system so as to more vigorously shield the body from infection or the development of maladies such as cancer. However, rigorous testing of these claims is typically lacking and so the claims of nutritional links with immune system improvement are at present tenuous.

A firmer link exists between exercise and immunomodulation. Moderately active people are known to have macrophages that are more capable of killing tumors, due to the increased production of a compound called nitric oxide. This population also displays lower incidence rates for cancer and other chronic diseases. Even sporadic exercise increases the ability of an immune system component called natural killer cells to eradicate tumors.

Conversely, too much exercise is associated with increased susceptibility to respiratory tract infections, indicating that the immune system is impaired in the ability to thwart infections.

Immunomodulation by microorganisms is directed at several aspects of the immune system. One target are the small molecules known as **cytokines**, which function as messengers of the immune system. In other words, cytokines stimulate various immune responses such as **inflammation** of the manufacture of antibodies. Other cytokines are involved in down-regulating the immune responses.

Some microorganisms are able to produce and excrete proteins that mimic the structure and function of cytokines. Often the result is a suppression of the host's inflammatory response. Examples of microbes that produce cytokine-like molecules are the **Epstein-Barr virus**, poxvirus, vaccinia virus.

Other microbes, such as the protozoan *Trypanosoma cruzi* blocks the activation of cytokines by an as yet unknown mechanism. The result is a severe suppression of the immune system. **Adenoviruses** also block cytokine expression, at the level of **transcription**.

The manipulation of cytokine expression and action may also be exploited to produce vaccines. For example, vaccines designed to nullify or enhance the activity of certain cytokines could cause greater activity of certain components of the immune system. While vaccines have yet to achieve this level of activity, specific experimental targeting of **deoxyribonucleic acid** has suppressed certain cytokines.

Another portion of the immune system capable of immunomodulation is **complement**. **Herpes** simplex virus types 1 and 2, the **viruses** responsible for cold sores and genital herpes in humans, resist the action of complement. The presence of specific viral proteins are required, and may act by disrupting a key enzyme necessary for complement manufacture.

Vaccinia virus can also evade complement action, via a protein that structurally resembles a host protein to which complement binds. Also, another viral protein, called the inflammation modulatory protein, acts to decrease the inflammatory response at the site of infection, thus preserving host tissue from damage and providing the virus particles with relatively undamaged cells in which to grow.

The protozoan *Trypanosoma cruzi* can regulate the activity of complement before infecting human cells. Once inside the cells of the host, the parasite can evade an immune response.

A variety of **bacteria**, viruses, and **parasites** are also able to modulate the immune system by affecting the way antigens are exposed on their surfaces. **Antigen** presentation is a complex series of steps. By controlling or modulating even one of these steps, the antigen presentation process can be disrupted. The formation of **antibody** is thus affected.

Aside from biological agents, physiological factors can cause immunomodulation. For example, stress is known to be capable of suppressing various aspects of the cellular immune response. The release of various hormones may disrupt in the normal expression of cytokines. Specifically, those cytokines that suppress inflammation are more evident, either because of their increased production or the decreased production of cytokines that activate inflammation.

See also Immunologic therapies

IMMUNOPRECIPITATION

• *see* ANTIBODY-ANTIGEN, BIOCHEMICAL AND MOLECULAR REACTIONS

IMMUNOSUPPRESSANT DRUGS

Immunosuppressant drugs are medications that reduce the ability of the **immune system** to recognize and respond to the presence of foreign material. Such drugs were developed and still have an important use as a means of ensuring that transplanted organs and tissues are not rejected by the recipient.

Rejection of transplanted organs or tissue is a natural reaction of a person's immune system. In a very real sense, the transplanted material is foreign and is treated, as would be an infectious microorganism. The immune system attacks and tries to destroy the foreign matter. Suppressing the immune system allows the transplanted material to be retained.

Drugs to suppress the immune system are available only with a physician's authorization. Some commonly prescribed drugs are azathioprine, cyclosporine, prednisolone, and tacrolimus. These can be taken orally, both in solid and liquid forms, or can be injected.

The main target of such immunosuppressant drugs are the white blood cells (which are also called lymphocytes). The main function of lymphocytes is to patrol the body and root out foreign material. Then these cells, in combination with other immune system components, destroy the foreign material.

Transplantation of animal kidneys into humans was tried in the early 1900s, and human-to-human transplant attempts were first made in 1933. These attempts were unsuccessful. It was not until the years of World War II that the immunological basis for these failures was deciphered. Then, **Peter Medawar** observed that a skin graft survived about a week before being rejected, but a subsequent graft was

rejected much more quickly. This led him to propose that an immunological response was at play in the rejection of transplanted material. This led to the first successful transplant in 1954, when the kidney of one identical twin was transplanted to the other twin. In the twins, the absence of genetic differences in their tissues would eliminate an immunological response.

As the role of the immune system in transplantation failure became more clear, the use of compounds to suppress the immune system began in the 1960s. In the 1960s and 1970s, the antigenic basis of immune recognition of foreign and nonforeign tissue became evident. With these discoveries came the recognition that the suppression of the immune system could aid in maintaining transplanted tissue. Successful transplantation of the liver was achieved in 1963, of the heart and small bowel in 1967.

In the 1980s, cyclosporin was discovered and shown to be effective in maintaining transplanted material. The clinical use of cyclosporin became standard. By the end of that decade, the use of immunosuppressant drugs just prior to and forever after a transplant had boosted the one-year transplant success rate to more than 80 per cent for all transplants except for the small intestine. In the present day, the survival rate of a kidney transplant is 86 percent even after five years.

Immunosuppressant drugs have other uses as well. Suppressing the immune system can lessen the disfigurement caused by severe forms of skin disorders such as psoriasis. Other examples include rheumatoid arthritis, Crohn's disease (which is an ongoing **inflammation** of the intestinal tract) and alopecia areata (nonuniform hair loss). In such cases the use of immunosuppressant therapy needs to be evaluated carefully, especially when the condition is not life threatening. This is because the deliberate suppression of the immune system can leave the individual vulnerable to other infections. Also, the clotting of blood can be inhibited, which could produce uncontrolled bleeding.

Another potential risk in the use of immunosuppressant drugs involves the administration of vaccines. The use of vaccines is not advisable when immunosuppressant drugs are being used, especially vaccines that utilize living but weakened **bacteria** or a virus as the agent designed to elicit protection. The deliberately immunocompromised individual could develop the disease for which the **vaccine** is intended to prevent.

The same risk analysis applies to the possible side effects of immunosuppressant drugs, which can include a higher than normal risk of developing some kinds of cancer later in life. The link between immunosuppressant drugs and cancer is not yet clear. The link was assumed to be a consequence of the interference with the ability of the body to detect and respond to cancerous cells. Conversely, cancer development has been viewed as being due partially to a failure of the immune system. Yet people with acquired **immunodeficiency** system, whose immune systems are also compromised, do not show increased rates of cancer. Instead, immunosuppressant drugs such as cyclosporine may themselves encourage the development of cancer by activating a cellular factor that makes cells more invasive.

It is now well known that the deliberate suppression of the immune system carries risks. However, the risks of a side effect or developing another illness, is usually less than the immediate health risk associated with not suppressing the immune system.

See also Autoimmunity and autoimmune diseases; Immunodeficiency

IN VITRO AND *IN VIVO* RESEARCH

In vitro research is generally referred to as the manipulation of organs, tissues, cells, and biomolecules in a controlled, artificial environment. The characterization and analysis of biomolecules and biological systems in the context of intact organisms is known as *in vivo* research.

The basic unit of living organisms is the cell, which in terms of scale and dimension is at the interface between the molecular and the microscopic level. The living cell is in turn divided into functional and structural domains such as the **nucleus**, the **cytoplasm**, and the secretory pathway, which are composed of a vast array of biomolecules. These molecules of life carry out the chemical reactions that enable a cell to interact with its environment, use and store energy, reproduce, and grow. The structure of each biomolecule and its subcellular localization determines in which chemical reactions it is able to participate and hence what role it plays in the cell's life process. Any manipulation that breaks down this unit of life, that is, the cell into its non-living components is, considered an *in vitro* approach. Thus, *in vitro,* which literally means "in glass," refers to the experimental manipulation conducted using cell-free extracts and purified or partially purified biomolecules in test tubes. Most of the biochemical and molecular biological approaches and techniques are considered genetic manipulation research. Molecular **cloning** of a **gene** with the aim of expressing its protein product includes some steps that are considered *in vitro* experiments such as the **PCR** amplification of the gene and the ligation of that gene to the expression vector. The expression of that gene in a host cell is considered an *in vivo* procedure. What characterizes an *in vitro* experiment is in principle the fact the conditions are artificial and are reconstructions of what might happen *in vivo*. Many *in vitro* assays are approximate reconstitutions of biological processes by mixing the necessary components and reagents under controlled conditions. Examples of biological processes that can be reconstituted *in vitro* are enzymatic reactions, folding and refolding of proteins and **DNA**, and the replication of DNA in the PCR reaction.

The definition of *in vitro* and *in vivo* research depends on the experimental model used. Microbiologists and **yeast** geneticists working with single cells or cell populations are conducting *in vivo* research while an immunologist who works with purified lymphocytes in tissue **culture** usually considers his experiments as an *in vitro* approach. The *in vivo* approach involves experiments performed in the context of the large system of the body of an experimental animal. In the case of *in vitro* fertilization, physicians and reproductive biologists

are manipulating living systems, and many of the biological processes involved take place inside the living egg and sperm. This procedure is considered an *in vitro* process in order to distinguish it from the natural fertilization of the egg in the intact body of the female.

In vivo experimental research became widespread with the use **microorganisms** and animal models in genetic manipulation experiments as well as the use of animal models to study drug toxicity in pharmacology. Geneticists have used prokaryotic, unicellular **eukaryotes** like yeast, and whole organisms like *Drosophila*, frogs, and mice to study genetics, **molecular biology** and toxicology. The function of genes has been studied by observing the effects of spontaneous **mutations** in whole organisms or by introducing targeted mutations in cultured cells. The introduction of gene cloning and *in vitro* mutagenesis has made it possible to produce specific mutations in whole animals thus considerably facilitating *in vivo* research. Mice with extra copies or altered copies of a gene in their genome can be generated by transgenesis, which is now a well-established technique. In many cases, the function of a particular gene can be fully understood only if a mutant animal that does not express the gene can be obtained. This is now achieved by gene knock-out technology, which involves first isolating a gene of interest and then replacing it *in vivo* with a defective copy.

Both *in vitro* and *in vivo* approaches are usually combined to obtain detailed information about structure-function relationships in genes and their protein products, either in cultured cells and test tubes or in the whole organism.

See also Immunogenetics; Immunologic therapies; Immunological analysis techniques; Laboratory techniques in immunology; Laboratory techniques in microbiology; Molecular biology and molecular genetics

INDICATOR SPECIES

Indicator organisms are used to monitor water, food or other samples for the possibility of microbial **contamination**. The detection of the designated species is an indication that harmful microbes, which are found in the same environment as the indicator species, may be present in the sample.

Indicator organisms serve as a beacon of fecal contamination. The most common fecal microorganism that is used have in the past been designated as fecal coliforms. Now, with more specific growth media available, testing for ***Escherichia coli*** can be done directly. The detection of *Escherichia coli* indicates the presence of fecal material from warm-blooded animals, and so the possible presence of disease producing **bacteria**, such as *Salmonella, Shigella,* and *Vibrio*.

To be an indicator organism, the bacteria must fulfill several criteria. The species should always be present in the sample whenever the bacterial pathogens are present. The indicator should always be present in greater numbers than the pathogen. This increases the chances of detecting the indicator. Testing directly for the pathogen, which can be more expensive and time-consuming, might yield a negative result if the numbers of the pathogen are low. Thirdly, the indicator bacterial species should be absent, or present in very low numbers, in clean water or other uncontaminated samples. Fourth, the indicator should not grow more abundantly than the pathogen in the same environment. Fifth, the indicator should respond to **disinfection** or **sterilization** treatments in the same manner as the pathogen does. For example, *Escherichia coli* responds to water disinfection treatments, such as **chlorination**, ozone, and ultra-violet irradiation, with the same sensitivity as does *Salmonella*. Thus, if the indicator organism is killed by the water treatment, the likelihood of *Salmonella* being killed also is high.

Another indicator bacterial species that is used are of the fecal *Streptococcus* group. These have been particularly useful in salt water monitoring, as they persist longer in the salt water than does *Escherichia coli*. In addition, the ratio of fecal coliform bacteria to fecal **streptococci** can provide an indication of whether the fecal contamination is from a human or another warm-blooded animal.

The use of indicator bacteria has long been of fundamental importance in the monitoring of drinking water. Similar indicator organisms will be needed to monitor water against the emerging protozoan threats of **giardia** and **cryptosporidium**.

See also Antibiotic resistance, tests for; Water quality

INDUSTRIAL MICROBIOLOGY • *see* ECONOMIC USES AND BENEFITS OF MICROORGANISMS

INFECTION AND RESISTANCE

Infection describes the process whereby harmful **microorganisms** enter the body, multiply, and cause disease. Normally the defense mechanisms of the body's **immune system** keep infectious microorganisms from becoming established. Those organisms, however, that can evade or diffuse the immune system and therapeutic strategies (e.g., the application of **antibiotics**) are able to increase their population numbers faster than they can be killed. The population increase usually results in host illness.

There are a variety of ways by which harmful microorganisms can be acquired. Blood contaminated with microbes, such as the viral agents of **hepatitis** and acquired **immunodeficiency** syndrome, is one source. Infected food or water is another source that causes illness and death to millions of people around the world every year. A prominent example is the food and water-borne transmission of harmful strains of ***Escherichia coli*** **bacteria**. Harmful microbes can enter the body through close contact with infected creatures. Transmission of the **rabies** virus by an infected raccoon bite and of encephalitis virus via mosquitoes are but two examples. Finally, breathing contaminated air can cause illness. Bacterial spores of the causative bacterial agent of **anthrax** are readily aerosolized and inhaled into the lungs, where, if sufficient in

A group of people with leprosy in the Middle East.

large enough numbers, can germinate and cause severe illness and even death.

To establish an infection, microbes must defeat two lines of defense of the body. The first line of defense is at body surfaces that act as a barrier guard the boundaries between the body and the outside world. These barriers include the skin, mucous membranes in the nose and throat, and tiny hairs in the nose that act to physically block invading organisms. Organisms can be washed away from body surfaces by tears, bleeding, and sweating. These are non-specific mechanisms of resistance.

The body's second line of defense involves the specific mechanisms of the immune system, a coordinated response involving a variety of cells and protein antibodies, whereby an invading microorganism is recognized and destroyed. The immune system can be strengthened by **vaccination**, which supplies or stimulates the creation of antibodies to an organism that the body has not yet encountered.

An increasing cause of **bacterial infection** is the ability of the bacteria to resist the killing action of antibiotics. Within the past decade, the problem of antibiotic resistant bacteria has become a significant clinical issue. Part of the reason for the development of resistance has been the widespread and sometimes inappropriate use of antibiotics (e.g., use of antibiotics for viral illness because antibiotics are not effective against **viruses**).

Resistance can have molecular origins. The membrane(s) of the bacteria may become altered to make entry of the antibacterial compound more difficult. Also, **enzymes** can be made that will destroy or inactivate the antibacterial agent. These resistance mechanisms can be passed on to subsequent generations of bacteria that will then be able to survive in increasing numbers.

Bacteria can also acquire resistance to antibiotics and other antibacterial agents, even components of the immune system, by growing on body surfaces, passages, and tissues. In this mode of growth, termed a biofilm, the bacteria are enmeshed in a sticky polymer produced by the cells. The polymer and the slow, almost dormant, growth rate of the bacteria protect them from antibacterial compounds that would otherwise kill them, and can encourage the bacteria to become

resistant to the compounds. Examples of such resistance includes the chronic *Pseudomonas aeruginosa* lung infections experienced by those with cystic fibrosis and infection of artificially implanted material, such as urinary catheters and heart pacemakers.

Bacteria and viruses can also evade immune destruction by entering host cells and tissues. Once inside the host structures they are shielded from immune recognition.

See also Antibody and antigen; Antibody formation and kinetics; Antibody-antigen, biochemical and molecular reactions; Bacteria and bacterial infection; Bacterial adaptation; Biofilm formation and dynamic behavior; Immune system; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunodeficiency; Microbiology, clinical; Viruses and responses to viral infection

INFECTION CONTROL

Microorganisms are easily transmitted from place to place via vectors such as insects or animals, by humans that can harbor the infectious organism and shed them to the environment, and via movement through the air (in the case of some **bacteria**, **yeast**, and **viruses**). Microorganisms can adapt to antimicrobial treatments (the best example being the acquisition of inheritable **antibiotic resistance** by bacteria). Thus, the potential for the spread of infection by disease-causing microbes is substantial unless steps are taken to limit the spread. Such strategies are collectively termed infection control.

For many microorganisms, particularly bacteria, contact transmission is a common means of spread of infection. This can involve the fecal-oral route, where hands soiled by exposure to feces are placed in the mouth. Day care workers and the infants under their charge are a significant focus of such ***Escherichia coli*** infections. As well, touching a contaminated inanimate surface is a means of transmitting an infectious microorganism.

The contact route of transmission is the most common route in the hospital setting. Various steps can be taken to control the spread of infection through contact with contaminated surfaces. Proper handwashing, in fact, is the single most effective means of preventing the spread of infection. Thorough handwashing prevents spread of bacteria to others and also prevents **contamination** of work or food preparation surfaces.

The operating theatre is an example of a place where the importance of infection control measures is apparent. In the nineteenth century, before the importance of hygienic procedures was recognized, operations were used as a last resort because of the extremely high mortality rate after surgery. Pioneering efforts by scientists such as **Joseph Lister** made operating rooms much cleaner, which resulted in a drop in the death rate attributable to surgically acquired infections. In the present day, operating rooms are places where personal **hygiene** is meticulous, instruments and clothing is sterile, and where post-operative clean up is scrupulous.

In hospitals and particularly in research settings, the control of infections involves the use of filters that can be placed in the ventilation systems. Such filters prevent the movement of particles even as small as viruses from a containment area to other parts of a building. Work surfaces are kept free of clutter and are exposed to disinfectant both before and after work with microbes, to kill any transient organisms that may be on the inanimate surface. Laboratories often contain containment structures called fume hoods, in which organisms can be worked with isolated from the airflow of the remainder of the lab. Even the nature of the work surface is designed to thwart infection. Surfaces are constructed so as to be very smooth and to be watertight. The presence of crevasses and cracks at the junction between surfaces are ideal spots for the collection and breeding of infectious microorganisms.

Some infectious microorganisms can be transferred by animal or insect vectors. One example is the viral agent of **Yellow Fever**, which is transmitted to humans via the mosquito. Control of such an infection can be challenging. Typically a concerted campaign to kill the breeding population of the vector is required, along with measures to protect people from those vectors that might escape the eradication campaign. To use the Yellow Fever example, spraying in mosquito breeding sites could be supplemented with the use of mosquito netting over the beds of people in particularly susceptible regions.

Another strategy of infection control is the use of antimicrobial or antiviral agents in an effort to either defeat an infection or, in the case of vaccines, to protect against the spread of an infection. **Antibiotics** are an antimicrobial agent. They have been in common use for less than 75 years, and already history is showing that antibiotics achieve success but that this success should not be assumed to be everlasting. Bacteria are proving to be adept at acquiring resistance to many antibiotics. Indeed, already strains of enterococci and *Staphylococcus aureus* are known to be resistant to virtually all antibiotics currently in use.

Immunization against infection is a widely practiced and successful infection control strategy. Depending upon the target microbe, the **vaccination** program may be undertaken to prevent the seasonal occurrence of a malady such as **influenza**, or to eradicate the illness on a worldwide scale. An example of the latter is the World Health Organization's effort to eradicate polio.

One breeding ground for the development of resistant microbial populations is the hospital. Antibiotics and disinfectants are an important part of the infection control strategy in place in most hospitals. Bacteria are constantly exposed to antibacterial agents. The pressure to adapt is constant.

The degree of infection control is tailored to the institution. For example, in a day care facility, the observance of proper hygiene and proper food preparation may be adequate to protect staff and children. However, in a hospital or nursing home, where people are frequently immunocompromised, additional measures need to be taken to ensure that microbes do not spread. Such measures can include regular **disinfection** of surfaces, one-time use of specific medical equipment such as disposable needles, and well-functioning ventilation systems.

Young children lying on beds in a hospital ward.

The focus of infection control strategies has shifted with the emerging knowledge in the 1970s and 1980s of the existence and medical relevance of the adherent bacterial populations known as biofilms. These adherent growths can remain viable on surfaces after being treated with concentrations of chemicals that swiftly kill their free-floating counterparts. Infection control in areas such as physician and dentist offices, now focus on ensuring that equipment is free from biofilms, because the bacteria could be easily transferred from the equipment to a patient.

See also Bacteria and bacterial infection; Disinfection and disinfectants; Epidemics and pandemics; Hygiene

INFLAMMATION

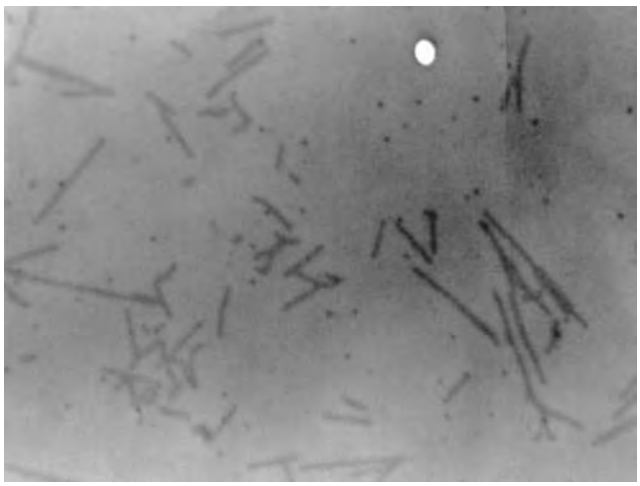
Inflammation is a localized, defensive response of the body to injury, usually characterized by pain, redness, heat, swelling, and, depending on the extent of trauma, loss of function. The process of inflammation, called the inflammatory response, is a series of events, or stages, that the body performs to attain homeostasis (the body's effort to maintain stability). The body's inflammatory response mechanism serves to confine, weaken, destroy, and remove **bacteria**, toxins, and foreign material at the site of trauma or injury. As a result, the spread of invading substances is halted, and the injured area is prepared for regeneration or repair. Inflammation is a nonspecific defense mechanism; the body's physiological response to a superficial cut is much the same as with a burn or a **bacterial infection**. The inflammatory response protects the body against a variety of invading pathogens and foreign matter, and should not be confused with an immune response, which reacts to specific invading agents. Inflammation is described as acute or chronic, depending on how long it lasts.

Within minutes after the body's physical barriers, the skin and mucous membranes, are injured or traumatized (for example, by bacteria and other **microorganisms**, extreme heat or cold, and chemicals), the arterioles and capillaries dilate, allowing more blood to flow to the injured area. When the blood vessels dilate, they become more permeable, allowing plasma and circulating defensive substances such as antibodies, phagocytes (cells that ingest microbes and foreign substances), and fibrinogen (blood-clotting chemical) to pass through the vessel wall to the site of the injury. The blood flow to the area decreases and the circulating phagocytes attach to and digest the invading pathogens. Unless the body's defense system is compromised by a preexisting disease or a weakened condition, healing takes place. Treatment of inflammation depends on the cause. Anti-inflammatory drugs such as aspirin, acetaminophen, ibuprofen, or a group of drugs known as NSAIDs (non-steroidal anti-inflammatory drugs) are sometimes taken to counteract some of the symptoms of inflammation.

An example of inflammation, showing the rash associated with hives.

INFLUENZA

Influenza (commonly known as flu) is a highly contagious illness caused by a group of **viruses** called the orthomyx-



Microscopic view of Infuenza virus.

oviruses. Infection with these viruses leads to a self-limiting illness usually characterized by fever, muscle aches, fatigue, and upper respiratory infection and **inflammation**. Children and young adults usually recover from influenza within 3–7 days with no complications; however, in older adults, especially those over 65 with underlying conditions such as heart disease or lung illnesses, influenza can be deadly. Most of the hospitalizations and deaths from influenza occur in this age group. Although an influenza **vaccine** is available, it does not confer complete protection against all strains of influenza viruses.

Like all viruses, orthomyxoviruses cause illness by entering host cells and replicating within them. The new viruses then burst from the host cell and infect other cells. Orthomyxoviruses are sphere-shaped viruses that contain **ribonucleic acid** (**RNA**). The viruses use this RNA as a blue-print for replication within host cells. The outer envelope of an orthomyxovirus is studded with protein spikes that help the virus invade host cells. Two different types of spikes are present on the virus's outer envelope. One type, composed of **hemagglutinin** protein (HA), fuses with the host cell membrane, allowing the virus particle to enter the cell. The other type of spike, composed of the protein neuraminidase (NA), helps the newly formed virus particles to bud out from the host cell membrane.

The only way a virus can be neutralized and stopped is through the body's immune response. At the present time, no cure or treatment is available that completely destroys viruses within the body. The HA spikes and proteins in the orthomyxovirus envelope stimulate the production of antibodies, immune proteins that mark infected cells for destruction by other immune cells. In a healthy person, it takes about three days for antibodies to be formed against an invading virus. People with impaired immune function (such as people with Acquired Immune Deficiency Syndrome, the elderly, or people with underlying conditions) may not be able to mount an effective immune response to the influenza virus. Therefore, these people may develop serious complications, such as **pneumonia**, that may lead to hospitalization or death.

Three types of orthomyxoviruses cause illness in humans and animals: types A, B, and C. Type A causes epidemic influenza, in which large numbers of people become infected during a short period of time. Flu **epidemics** caused by Type A orthomyxoviruses include the worldwide outbreaks of 1918, 1957, 1968, and 1977. Type A viruses infect both humans and animals and usually originate in the Far East, where a large population of ducks and swine incubate the virus and pass it to humans. The Far East also has a very large human population that provides a fertile ground for viral replication. In 1997, a new strain of influenza A jumped from the poultry population in Hong Kong to the human population. H5N1, as the strain was named, was contracted through contact with the feces of chicken. The illness it caused (dubbed avian flu) was severe, and sometimes fatal. Although it was strongly believed that humans could not get the disease from eating properly cooked chicken, the decision was ultimately made to destroy and bury all of the chickens in Hong Kong. This massive effort was carried out in December 1997.

Type B influenza viruses are not as common as type A viruses. Type B viruses cause outbreaks of influenza about every two to four years. Type C viruses are the least common type of influenza virus and cause sporadic and milder infections.

The hallmark of all three kinds of influenza viruses is that they frequently mutate. Due to the small amount of RNA genetic material within a virus, mutation of the genetic material is very common. The result of this frequent mutation is that each flu virus is different, and people who have become immune to one flu virus are not immune to other flu viruses. The ability to mutate frequently therefore allows these viruses to cause frequent outbreaks.

Influenza is characterized by a sudden onset of fever, cough, and malaise. The incubation period of influenza is short, only 1–3 days. The cells that the influenza virus target are the cells of the upper respiratory tract, including the sinuses, bronchi, and alveoli. The targeting of the upper respiratory tract by the viruses accounts for the prominence of respiratory symptoms of flu. In fact, flu viruses are rarely found outside the respiratory tract. Most of the generalized symptoms of flu, such as muscle aches, are probably due to toxin-like substances produced by the virus.

Symptoms last for about 3–6 days; however, lethargy and cough may persist for several days to weeks after a bout with the flu. Children may have more severe symptoms due to a lack of general **immunity** to influenza viruses. Children also have smaller airways, and thus may not be as able to compensate for respiratory impairment as well as adults.

The most common complication of influenza is pneumonia. Pneumonia may be viral or bacterial. The viral form of pneumonia that occurs with influenza can be very severe. This form of pneumonia has a high mortality rate. Another form of pneumonia that is seen with influenza is a bacterial pneumonia. If the respiratory system becomes severely obstructed during influenza, **bacteria** may accumulate in the lungs. This type of pneumonia occurs 5–10 days after onset of the flu. Because it is bacterial in origin, it can be treated with **antibiotics**.

Other complications of influenza include infections of the heart and heart lining, infections of the brain, and Guillain-Barre syndrome (GBS). GBS is a paralytic disease in which the body slowly becomes paralyzed. Paralysis starts in the facial muscles and moves downward. GBS is treated symptomatically and usually resolves by itself. Another complication of influenza is Reye's syndrome. Occurring typically in children, Reye's syndrome is associated with aspirin intake during an attack of influenza. Reye's syndrome is characterized by nausea, vomiting, and progressive neurological dysfunction. Because of the risk of Reye's syndrome, children should not be given aspirin if they have the flu. Non-aspirin pain relievers, such as acetaminophen, should be given instead of aspirin.

Flu is treated with rest and fluids. Maintaining a high fluid intake is important, because fluids increase the flow of respiratory secretions, which may prevent pneumonia. Antiviral medications (amantadine, rimantadine) may be prescribed for people who have initial symptoms of the flu and who are at high risk for complications. This medication does not prevent the illness, but reduces its duration and severity.

A flu vaccine is available that is formulated each year against the current type and strain of flu virus. The virus is grown in chicken eggs, extracted, and then rendered noninfective by chemicals. The vaccine is also updated to the current viral strain by the addition of proteins that match the current strain's composition. The vaccine would be most effective in reducing attack rates if it was effective in preventing influenza in schoolchildren; however, in vaccine trials the vaccine has not been shown to be effective in flu prevention in this age group. In certain populations, particularly the elderly, the vaccine is effective in preventing serious complications of influenza and thus lowers mortality.

Vaccine research is ongoing. One of the more exciting advances in flu vaccines involves research studies examining an influenza vaccine mist, which is sprayed into the nose. This is predicted to be an excellent route of administration, which will confer even stronger immunity against influenza. Because it uses a live virus, it encourages a strong immune response. Furthermore, it is thought to be a more acceptable **immunization** route for schoolchildren, an important reservoir of the influenza virus.

See also Flu: The great flu epidemic of 1918; Viruses and responses to viral infection

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Interferon actions

Interferons are species-specific proteins, which induce antiviral and antiproliferative responses in animal cells. They are a major defense against viral infections and abnormal growths (neoplasms). Interferons are produced in response to penetration of animal cells by viral (or synthetic) nucleic acid and then leave the infected cell to confer resistance on other cells of the organism. In contrast to antibodies, interferons are not virus specific but host specific. Thus, viral infections of human cells are inhibited only by human interferon. The human genome contains 14 nonallelic and 9 allelic genes of α-interferon (macrophage interferon), as well as a single **gene** for β-interferon (fibroblast interferon). Genes for any two or more variants of interferon, which have originated from the same wild-type gene are called allelic genes and will occupy the same chromosomal location (locus). Variants originating from different standard genes are termed non allelic. α- and β-interferons are structurally related glycoproteins of 166 and 169 amino acid residues. In contrast, γ-interferon (also known as immune interferon) is not closely related to the other two and is not induced by virus infection. It is produced by T-cells after stimulation with the cytokine interleukin-2. It enhances the cytotoxic activity of T-cells, macrophages and natural killer cells and thus has antiproliferative effects. It also increases the production of antibodies in response to antigens administered simultaneously with α-interferon, possible by enhancing the antigen-presenting function of macrophages.

Interferons bind to specific receptors on the cell surface, and induce a signal in the cell interior. Two induction mechanisms have been elucidated. One mechanism involves the induction of protein kinase by interferon, which, in the presence of double-stranded **RNA**, phosphorylates one subunit of an initiation factor of **protein synthesis** (eIF-2B), causing the factor to be inactivated by sequestration in a complex. The second mechanism involves the induction of the enzyme 2',5'-oligoadenylate synthetase (2',5'-oligo A synthetase). In the presence of double-stranded RNA, this enzyme catalyses the polymerization of ATP into oligomers of 2 to 15 adenosine monophosphate residues which are linked by phosphodiester bonds between the position 2' of one ribose and 5' of the next. These 2',5'-oligoadenylates activate an interferon specific RNAase, a latent endonuclease known as RNAase L which is always present but not normally active. RNAase cleaves both viral and cellular single stranded mRNA. Interferons therefore do not directly protect cells against viral infection, but rather render cells less suitable as an environment for viral replication. This condition is known as the antiviral state.

See also Antibody and antigen; Immune system; Immunology; Viruses and responses to viral infection

Interferons

Interferon is the name given to a group of proteins known primarily for their role in inhibiting viral infections and in stimulating the entire **immune system** to fight disease. Research has also shown that these proteins play numerous roles in regulating many kinds of cell functions. Interferons can promote or hinder the ability of some cells to differentiate, that is, to become specialized in their function. They can inhibit cell division, which is one reason why they hold promise for stopping cancer growth. Recent studies have also found that one interferon may play an important role in the early biological

processes of pregnancy. Although once thought to be a potential cure-all for a number of viral diseases and cancers, subsequent research has shown that interferons are much more limited in their potential. Still, several interferon proteins have been approved as therapies for diseases like chronic **hepatitis**, genital warts, multiple sclerosis, and several cancers.

The first interferon was discovered in 1957 by Alick Isaacs and Jean Lindenmann. During their investigation, the two scientists found that virus-infected cells secrete a special protein that causes both infected and noninfected cells to produce other proteins that prevent **viruses** from replicating. They named the protein interferon because it interferes with infection. Initially, scientists thought there was only one interferon protein, but subsequent research showed that there are many different interferon proteins.

Interferons are members of a larger class of proteins called **cytokines** (proteins that carry signals between cells). Most interferons are classified as alpha, beta, or gamma interferons, depending on their molecular structure. Two other classes of interferons, omega and tau, have also been discovered. So far, more than 20 different kinds of interferon-alpha have been discovered but few beta and gamma interferons have been identified.

Interferons are differentiated primarily through their amino acid sequence. (Amino acids are molecular chains that make up proteins.) Interferon-alpha, -beta, -tau, and -omega, which all have relatively similar amino acid sequences, are classified as type I interferons. Type I interferons are known primarily for their ability to make cells resistant to viral infections. Interferon-gamma is the only type II interferon, classified as such because of its unique amino acid sequence. This interferon is known for its ability to regulate overall immune system functioning.

In addition to their structural makeup, type I and type II interferons have other differences. Type I interferons are produced by almost every cell in the body, while the type II interferon-gamma is produced only by specialized cells in the immune system known as T lymphocytes and natural killer cells. The two classes also bind to different kinds of receptors, which lie on the surface of cells, and attract and combine with specific molecules of various substances.

Interferons work to stop a disease when they are released into the blood stream and then bind to cell receptors. After binding, they are drawn inside the cell's **cytoplasm**, where they cause a series of reactions that produce other proteins that fight off disease. Scientists have identified over 30 disease-fighting proteins produced by interferons.

In addition to altering a cell's ability to fight off viruses, interferons also control the activities of a number of specialized cells within the immune system. For example, type I interferons can either inhibit or induce the production of **B lymphocytes** (white blood cells that make antibodies for fighting disease). Interferon-gamma can also stimulate the production of a class of T lymphocytes known as suppressor CD8 cells, which can inhibit **B cells** from making antibodies.

Another role of interferon-gamma is to increase immune system functioning by helping macrophages, still another kind of white blood cell, to function. These scavenger cells attack infected cells while also stimulating other cells within the immune system. Interferon-gamma is especially effective in switching on macrophages to kill tumor cells and cells that have been infected by viruses, **bacteria**, and **parasites**.

Interferon-tau, first discovered for its role in helping pregnancy to progress in cows, sheep, and goats, also has antiviral qualities. It has been shown to block tumor cell division, and may interfere with the replication of the acquired immune deficiency, or **AIDS**, virus. Because it has fewer unwanted side-effects (flu-like symptoms and decreased blood cell production) than the other interferons, interferon-tau is becoming a new focal point for research.

In 1986, interferon-alpha became the first interferon to be approved by the Food and Drug Administration (FDA) as a viable therapy, in this case, for hairy-cell leukemia. (Interferons are used therapeutically by injecting them into the blood stream.) In 1988, this class of interferons was also approved for the treatment of genital warts, proving effective in nearly 70% of patients who do not respond to standard therapies. In that same year, it was approved for treatment of Kaposi's Sarcoma, a form of cancer that appears frequently in patients suffering from AIDS. In 1991, interferon-alpha was approved for use in chronic hepatitis C, a contagious disease for which there was no reliable therapy. Interferon has been shown to eliminate the disease's symptoms and, perhaps, prevent relapse. Interferon-alpha is also used to treat Hodgkin's lymphoma and malignant melanoma.

In 1993, another class of interferon, interferon-gamma, received FDA approval for the treatment of a form of multiple sclerosis characterized by the intermittent appearance and disappearance of symptoms. It has also been used to treat chronic granulomatous diseases, inherited immune disorders in which white blood cells fail to kill bacterial infections, thus causing severe infections in the skin, liver, lungs, and bone. Interferon-gamma may also have therapeutic value in the treatment of leishmaniasis, a parasitic infection that is prevalent in parts of Africa, America, Europe, and Asia.

Although all of the disease fighting attributes of interferon demonstrated in the laboratory have not been attained in practice, continued research into interferons will continue to expand their medical applications. For example, all three major classes of interferons are under investigation for treating a variety of cancers. Also, biotechnological advances making genetic engineering easier and faster are making protein drugs like interferons more available for study and use. Using recombinant **DNA** technology, or **gene** splicing, genes that code for interferons are identified, cloned, and used for experimental studies and in making therapeutic quantities of protein. These modern DNA manipulation techniques have made possible the use of cell-signaling molecules like interferons as medicines. Earlier, available quantities of these molecules were too minute for practical use.

See also AIDS, recent advances in research and treatment; Interferon actions; Viral genetics; Viral vectors in gene therapy; Virology; Virus replication; Viruses and responses to viral infection

INVASIVENESS AND INTRACELLULAR INFECTION

Microorganisms that establish infections in humans do so by a number of means. For example, some **bacteria** remain associated with the surface of host cells, but elaborate a coating that provides protection from host immune defenses and external antimicrobial agents, such as **antibiotics**. Another strategy that is used by a number of disease-causing bacteria, virtually all **viruses**, single-celled eukaryotic **parasites**, and **protozoa** is the invasion of the host cells to which the microorganisms adhere. Once inside the host cell, the invading microbe is shielded from host defenses and therapeutic antimicrobial compounds.

Following the invasion of host cells, the microorganisms can establish an infection inside the host cells. This is referred to as intracellular infection. Once again, by remaining inside the host, the bacteria or protozoa are shielded from attack.

Intracellular infection presents a problem for the host, since the infection cannot be dealt with without damage to the host's own tissue. Many disease-causing microorganisms have adopted this mode of infection, including all viruses, some protozoa, and some bacteria. Indeed, some of these intracellular parasites depend absolutely on this mode of growth and cannot survive outside of the host cells. Two examples are the bacteria *Chlamydia* and *Rickettsia*. These bacteria are transmitted to another host only by direct contact of host cells, such as in sexual activity, or when they are sucked up by a biting insect and subsequently expelled into another host.

The molecular nature of invasiveness has been well studied in a number of Gram-negative bacteria. One example is designated as enteropathogenic ***Escherichia coli***, or EPEC. This bacterium causes a severe, debilitating, and sometimes life-threatening diarrhea in infants, particularly in underdeveloped countries. EPEC associates with host epithelial cells in the intestinal tract by means of appendages known as fimbriae. Once adhesion is established, the bacterium produces a number of proteins that are then passed across the cell wall to the surface. Studies with **mutants** that do not manufacture one or more of these proteins have shown that the proteins are essential for invasion of the host cell. The exact function of these proteins in the invasive process is still unclear. But current data indicates that they have a role in altering the processes by which host cells transport compounds, and so may facilitate the movement of bacterial disease-causing compounds into the host cell. Finally, the bacteria form a protein that functions as an anchor, to irreversibly bind the bacterial cell to the host cell. Thus, while EPEC are not fully taken into the host cell, an intracellular invasion pathway is established.

A bona fide invasion of host cells is accomplished by the bacterium *Salmonella*. The bacteria have a number of genes, which are clustered together on the bacterial genome, which are activated following association of a bacterium with a host intestinal epithelial cell. The products of the genes operate in a similar fashion as those of EPEC. That is, they provide a conduit for the transport of bacterial compounds into the host cell. *Salmonella* additionally produces a protein that enters the host cell and modifies a scaffolding system in the cell that is called the actin cytoskeleton. The alteration is thought to cause the host cell membrane to become more pliable and capable of becoming much more wavy. This so-called ruffling can entrap a bacterium, enabling it to be taken into the host cell in a membrane-bound bag that is called a vacuole. Once inside the host, other proteins produced by the bacterium cause cell damage and allow the establishment of an infection. The bacteria remain inside the vacuole

Another Gram-negative species called *Shigella flexneri* also promotes the ruffling of the host membrane, which results in the uptake of the bacteria into the host cell. In contrast to *Salmonella*, *Shigella flexneri* break out of the vacuole and produce more copies of themselves in the cellular fluid of the host cell. In the host fluid a bacterium becomes coated with host molecules called actin. By propelling itself against the end of a host cell, a bacterium is able to use the stiff actin filament as a kind of battering ram, to punch a hole through to the neighbouring host cell. This enables the **bacterial infection** to spread from cell to cell without ever contact the surface of the host cells.

Other host cells can be invaded. For example, another Gram-negative bacterial species called *Legionella pneumophila* invades macrophages. Macrophages are white blood cells that are part of the **immune system**. By invading a macrophage, the bacteria can render the macrophage incapable of functioning in defending the body from infection. Thus, invasion serves not only to provide the bacteria with a safe haven for replication, but also compromises the immune system, facilitating the establishment of a bacterial infection.

Invasion of host cells and replication inside the cells can be a stage in the infectious cycle of microorganisms. For example, the single-celled eukaryotic parasites called *Entamoeba histolytica* and *Entamoeba dispar* can invade epithelial cells in the colon. Following the intracellular invasion the amoeba can become dispersed throughout the body via the bloodstream, leading to persistent infections, such as in the liver.

The protozoan called *Cryptosporidium parvum* causes a debilitating diarrhea, typically after being ingested in feces-contaminated drinking water. A key feature of the protozoan infection is the invasion of host epithelial cells in the ileum by a specialized form of the protozoan known as the sporozoite. Replication occurs inside the host cell with the progeny protozoa being released upon rupture of the host cell. The progeny can then go on to invade adjacent host tissue.

The wide spread distribution of host cell invasion and intracellular replication among microorganisms is indicative of the success of the strategy.

See also Bacteria and bacterial infection

ISOTYPES AND ALLOTYPES

Isotype and allotype are terms that relate to the structure of a component of the **immune system** that is called an immunoglobulin.

Immunoglobulins bind their corresponding **antigen**. An immunoglobulin can be static, as part of a membrane-bound receptor to which an antigen binds, or can be floating freely in

the body as an **antibody**. The antigen-binding capacity of an immunoglobulin is related to the three-dimensional shape of the molecule. Immunoglobulin isotype and allotype determines the diversity in shape of immunoglobulins.

Immunoglobulins are structured with two Y-shaped "heavy" chains. To each of these a shorter "light" chain is linked. Within each chain there are regions whose amino acid sequence remains constant from immunoglobulin to immunoglobulin. There are also regions whose amino acid sequence is somewhat different and markedly different between the myriad of immunoglobulins that can be made.

The type of heavy chain an immunoglobulin has determines the isotype. Immunoglobulins of the same isotype have the same amino acid structure in two specific regions of the heavy chain. The similarity in amino acid sequence extends to the three-dimensional structure that the immunoglobulin adopts. Thus, immunoglobulins of the same isotype have similar shapes and so similar antigen binding characteristics. The different classes and subclasses of antibody protein arising from the amino acid variations represent the different isotypes.

In humans, there are five immunoglobulin isotypes: IgA, IgD, IgG, IgE, and IgM. All humans whose immune system is functioning correctly possess all these isotypes. Thus, no antigenic response would be elicited if an IgA from one person was injected into another.

Immunoglobulins can also be classified by their so-called allotypes. Allotypes are also determined by the amino acid sequences of the heavy and light chains. Allotypes focus on the variable regions of the chains. By their variable nature, differences in the amino acid sequence can exist even between members of the same species. Allotypes represent what is termed polymorphisms within certain heavy or light chains.

Not all members of a species such as man possess any particular allotype. In contrast to isotypes, the injection of a specific allotype from one person into another could elicit an antigenic reaction in the recipient.

See also Antibody-antigen, biochemical and molecular reactions

IVANOVSKY, DMITRI IOSIFOVICH
(1864-1920)

Russian botanist

Dmitri Ivanovsky, in studying a disease that affects tobacco plants, paved the way for the discovery of the infectious particle known as a virus.

Ivanovsky, the son of a landowner, was born in Gdov, Russia. He attended the Gymnasium of Gdov and later graduated as a gold medalist from the Gymnasium of St. Petersburg in 1883. At the University of St. Petersburg, he enrolled in the natural science department and studied under several prominent Russian scientists. While a student, he became interested in diseases that destroy tobacco plants. He graduated in 1888 after presenting his thesis *On Two Diseases of Tobacco Plants.*

The following year, he was asked by the directors of the Department of Agriculture to study a new tobacco disease, called tobacco mosaic, that had afflicted plants in the Crimean region. He crushed the infected leaves, which were distinguished by their mosaic pattern, into sap and then forced the material through a Chamberland bacterial filter that was known to remove all **bacteria**. Despite following this procedure, the sap, when brushed on the leaves of healthy plants, was still toxic enough to cause disease. Ivanovsky's 1892 report on the tobacco mosaic disease detailed what he maintained must be an agent smaller than bacteria. It was the first study in which factual evidence was offered concerning the existence of this new kind of infectious pathogen.

Ivanovsky's work was ignored by the scientific community, and he eventually abandoned his study of this pathogen without understanding the implications of his research. The Dutch botanist, **Martinus Willem Beijerinck**, repeated Ivanovsky's experiments with this new pathogenic source, giving it the name filterable virus in 1898.

See also Tobacco mosaic virus (TMV); Virology

J

JACOB, FRANÇOIS (1920-)

French molecular biologist

François Jacob made several major contributions to the field of genetics through successful collaborations with other scientists at the famous Pasteur Institute in France. His most noted work involved the formulation of the Jacob-Monod **operon** model, which helps explain how genes are regulated. Jacob also studied messenger **ribonucleic acid** (mRNA), which serves as an intermediary between the **deoxyribonucleic acid** (**DNA**), which carries the **genetic code**, and the **ribosomes**, where proteins are synthesized. He also demonstrated that **bacteria** follow the same general rules of natural **selection** and **evolution** as higher organisms. In recognition of their work in genetic control and **viruses**, Jacob and two other scientists at the Pasteur Institute, **Jacques Lucien Monod** and André Lwoff, shared the 1965 Nobel Prize for Physiology or Medicine.

Jacob was born in Nancy, France, to Simon Jacob, a merchant, and the former Thérèse Franck. Jacob attended school at the Lycée Carnot in Paris before beginning his college education. He began his studies toward a medical degree at the University of Paris (Sorbonne), but was forced to cut his education short when the German Army invaded France during World War II in 1940. He escaped on one of the last boats to England and joined the Free French forces in London, serving as an officer and fighting with the Allies in northern Africa. During the war, Jacob was seriously wounded. His injuries impaired his hands and put an abrupt end to his hopes of becoming a surgeon. For his service to his country, he received the Croix de Guerre and the Companion of the Liberation, two of France's highest military honors.

Despite this physical setback, Jacob continued his education at the University of Paris. In his autobiography, *The Statue Within,* Jacob said he got the idea for his thesis from his place of work, the National **Penicillin** Center, where a minor antibiotic called tyrothricin was manufactured and commercialized. For his thesis, Jacob manufactured and evaluated the drug. Nearing thirty years old, he earned his M.D. degree in 1947, the same year he married Lysiane "Lise" Bloch, a pianist. They would eventually have four children.

With his professional future unsure, Jacob continued to work for a while at the National Penicillin Center. The tide turned when he and his wife had dinner with her cousins, including Herbert Marcovich, a biologist working in a genetics lab. Jacob recalled, "As Herbert spoke, I felt an excitement rising like a storm. If a man of my generation could still go into research without making himself ridiculous, then why not I?" He decided to become a biologist the next day.

Jacob joined the Pasteur Institute in 1950 as an assistant to André Lwoff. Lwoff's laboratory location and its cramped quarters earned it the name of "the attic." The year 1950 was an exciting one in Lwoff's lab. Lwoff had been working with lysogenic bacteria, which are destroyed (lysed) when attacked by bacteria-infecting virus particles called bacteriophages. The bacteriophages invade the bacterial cell, then multiply within it, eventually bursting the cell and releasing new bacteriophages. According to Lwoff's research, the **bacteriophage** first exists in the bacterial cell in a non-infectious phase called the prophage. He could stimulate the prophage to begin producing infective virus by adding ultraviolet light. These new findings helped to give Jacob the background he would need for his future research.

Jacob continued his education at the University of Paris during his first years at the Pasteur Institute, earning his bachelor of science in 1951 and studying toward his doctor of science degree, which he received in 1954. For his doctoral dissertation, Jacob reviewed the ability of certain radiations or chemical compounds to inducethe prophage, and proposed possible mechanisms of **immunity**.

Once on staff in the lab, Jacob soon formed what would become a fruitful collaboration with Élie Wollman, also stationed in Lwoff's laboratory. In the summer of 1954 he and Wollman discovered what they termed erotic induction in the bacteria *Escherichia coli*. They later changed the name of the phenomenon to zygotic induction. In zygotic induction, the chromosome of a male bacterial cell carrying a prophage

François Jacob, whose research on the operon culminated in a Nobel Prize.

could be transferred to a female cell which was not carrying the prophage, but not vice versa. Zygotic induction showed that both the expression of the prophage and immunity was blocked in the latter instance by a variable present in the **cytoplasm** that surrounds the cell's **nucleus**.

In another experiment, he and Wollman mated male and female bacterial cells, separating them before they could complete **conjugation**. This also clipped the chromosome as it was moving from the male to the female. They found that the female accepted the chromosome bit by bit, in a certain order and at a constant speed, rather similar to sucking up a piece of spaghetti. Their study became known as the "spaghetti experiment," much to Wollman's annoyance.

In the book *Phage and the Origins of Molecular Biology,* Wollman explained that by following different genetic markers in the male, they could determine each gene's time of entry into the zygote and correctly infer its position on the DNA. Jacob and Wollman also used an **electron microscope** to photograph the conjugating bacteria and time the transmission of the genes. "With Élie Wollman, we had developed a tool that made possible genetic analysis of any function, any "system," Jacob said in his autobiography. The two scientists also discovered and defined **episomes**, genetic strains which automatically replicate as part of the development of **chromosomes**.

Jacob and Wollman also demonstrated that bacteria could mutate and adapt in response to drugs or bacteriophages. Evolution and natural selection worked in bacteria as well as in higher life forms. Jacob and Wollman summarized their research in the July, 1956, issue of *Scientific American:* "There is little doubt that the basic features of genetic **recombination** must be similar whether they occur in bacteria or in man. It would be rather surprising if the study of sexual reproduction in bacteria did not lead to deeper understanding of the process of genetic recombination, which is so vital to the survival and evolution of higher organisms."

In 1956 Jacob accepted the title of laboratory director at the Pasteur Institute. Within two years Jacob began to work with Jacques Monod, who had left Lwoff's lab several years earlier to direct the department of cellular **biochemistry** at the Pasteur Institute. Arthur Pardée also often joined in the research. Jacob and Monod studied how an intestinal enzyme called galactosidase is activated to digest lactose, or milk sugar. Galactosidase is an inducible enzyme, that is, it is not formed unless a certain substrate—in this case lactose—is present. Inducible **enzymes** differ from constitutive enzymes which are continuously produced, whether or not the inducer is present. By pairing a normal inducible male bacteria with a constitutive female, they showed that inducible enzyme processes take precedence over constitutive enzyme synthesis. In the experiments conducted by Jacob and Monod, the inducer, lactose, served to inhibit the **gene** that was regulating the synthesis of galactosidase.

Afterward, Jacob realized that his work with Monod and his earlier work with Wollman on zygotic induction were related. In *The Statue Within,* he said, "In both cases, a gene governs the formation of a cytoplasmic product, of a repressor blocking the expression of other genes and so preventing either the synthesis of the galactosidase or the multiplication of the virus." Their chore then was to determine the location of the repressor, which appeared to be on the DNA.

By the end of the decade, Jacob and Monod had discovered messenger **RNA**, one of the three types of ribonucleic acid. (The other two are ribosomal RNA and transfer RNA.) Each type of RNA has a specific function. MRNA is the mediator between the DNA and ribosomes, passing along information about the correct sequence of amino acids needed to make up proteins. While their work continued, Jacob accepted a position as head of the Department of Cell Genetics at the Pasteur Institute.

In 1961, they explained the results of their research involving the mRNA and the now-famous Jacob-Monod operon model in the paper, "Genetic Regulatory Mechanisms in the Synthesis of Proteins," which appeared in the *Journal of Molecular Biology* Molecular biologist Gunther S. Stent in *Science* described the paper "one of the monuments in the literature of molecular biology."

According to the Jacob-Monod operon model, a set of structural genes on the DNA carry the code that the messenger RNA delivers to the ribosomes, which make proteins. Each set of structural genes has its own operator gene lying next to it. This operator gene is the switch that turns on or turns off its set of structural genes, and thus oversees the synthesis of their proteins. Jacob and Monod called each grouping of an operator and its structural genes an operon. Besides the operator gene, a regulator gene is located on the same chromosome as the structural genes. In an inducible system, like the lactose

operon (or lac operon as it is called), this regulator gene codes for a repressor protein. The repressor protein does one of two things. When no lactose is present, the repressor protein attaches to the operator and inactivates it, in turn, halting structural gene activity and **protein synthesis**. When lactose is present, however, the repressor protein binds to the regulator gene instead of the operator. By doing so, it frees up the operator and permits protein synthesis to occur. With a system such as this, a cell can adapt to changing environmental conditions, and produce the proteins it needs when it needs them.

A year after publication of this paper, Jacob won the Charles Leopold Mayer Prize of the French Academy of Sciences. In 1964, Collège de France also recognized his accomplishments by establishing a special chair in his honor. His greatest honor, however, came in 1965 when he, Lwoff, and Monod shared the Nobel Prize for Physiology or Medicine. The award recognized their contributions "to our knowledge of the fundamental processes in living matter which form the bases for such phenomena as adaptation, reproduction and evolution."

During his career, Jacob wrote numerous scientific publications, including the books *The Logic of Life: A History of Hereditary* and *The Possible and the Actual*. The latter, published in 1982, delves into the theory of evolution and the line that he believes must be drawn between the use of evolution as a scientific theory and as a myth.

See also Bacteriophage and bacteriophage typing; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Immunogenetics; Molecular biology and molecular genetics; Molecular biology, central dogma of; Viral genetics

JANNASCH, HOLGER WINDEKILDE (1927-1998)

German marine microbiologist

Holger Jannasch was a marine microbiologist who made fundamental contributions to the study of microbial life in the extreme environment of the deep-sea. His discoveries helped reveal a hitherto unknown type of **bacterial growth** and broadened human knowledge about the diversity of life on Earth.

Jannasch was born in Holzminden, Germany. After a short stint as a lumberjack, he returned to school. His educational experiences and a job as a warden at a coastal bird sanctuary stimulated an interest in both biological life and the ocean. These interests were pursued during graduate studies at the University of Göttingen. He received his Ph.D. in biology in 1955. From 1956 to 1960 he was an assistant scientist at the Max Planck Society. At the same time he was also a post-doctoral fellow at the Scripps Institution of Oceanography in San Diego, California, and at the University of Wisconsin. From 1961 to 1963 he served as an assistant professor in the Department of Microbiology at the University of Göttingen. He also held the position of Privatdozent at that University from 1963 until his death.

Visits to the Woods Hole Oceanographic Institution in the early 1960s lead to his joining the institution in 1963. He remained there for the remainder of his career and life.

While at Woods Hole, Jannasch proved to be a consummate mentor and educator. As well, he was a prolific researcher. His main interests were the growth of **microorganisms** in the sea, the existence of microbes at the low temperature and high pressure of the ocean depths, and the microbial processes taking place at **hydrothermal vents** on the ocean floor. Indeed, it was Jannasch who discovered hydrothermal vents.

Jannasch's research on the hydrothermal vents and their associated bacterial populations became classic papers that inspired other microbiologists to similar research. His discovery of sulfur-utilizing **bacteria** that support an entire hydrothermal ecosystem has had major implications for deep sea microbial ecology and may be of fundamental importance to providing insight into the **origin of life** on Earth.

Jannasch was also a seminal influence of the field of microbial ecology. He was a participating author on some 200 research publications. For these and other accomplishments in microbial ecology, a new microorganism was named for him in 1966: *Methanococcus jannaschii*. That same year Woods Hole established the Holger W. Jannasch Chair in recognition of his accomplishments.

Many other awards and honors were bestowed on Jannasch during his career. Foe example, in 1995 he was one of only a handful of non-United States citizens elected to the National Academy of Sciences.

See also Extremophiles

JENNER, EDWARD (1749-1823)

English physician

Edward Jenner discovered the process of **vaccination**, when he found that injection with **cowpox** protected against **smallpox**. His method of **immunization** via vaccination ushered in the new science of **immunology**.

Jenner was born in Berkeley, England, the third son and youngest of six children of Stephen Jenner, a clergyman of the Church of England. He was orphaned at age five and was raised by his older sister, who was married to a clergyman. When Jenner was thirteen years old, he was apprenticed to a surgeon. Then in 1770, he moved to London, England, to work with John Hunter (1728 – 1798), an eminent Scottish anatomist and surgeon who encouraged Jenner to be inquisitive and experimental in his approach to medicine. Jenner returned to Berkeley in 1773, and set up practice as a country doctor.

During and prior to Jenner's lifetime, smallpox was a common and often fatal disease worldwide. Many centuries before Jenner's time, the Chinese had begun the practice of blowing flakes from smallpox scabs up the nostrils of healthy persons to confer **immunity** to the disease. By the seventeenth century, the Turks and Greeks had discovered that, when injected into the skin of healthy individuals, the serum from the

Edward Jenner (right), innoculating a boy with cowpox virus as a protection against smallpox.

smallpox pustule induced a mild case of the disease and subsequent immunity. This practice of inoculation, termed variolation, reached England by the eighteenth century. However, it was quite risky as those who were inoculated frequently suffered a severe or fatal case of smallpox. Despite the risk, people willingly agreed to inoculation because of the widespread incidence of smallpox and the fear of suffering from terribly disfiguring pockmarks that resulted from the disease.

As a young physician, Jenner noted that dairy workers who had been exposed to cowpox, a disease like smallpox only milder, seemed immune to the more severe infection. He continually put forth his theory that cowpox could be used to prevent smallpox, but his contemporaries shunned his ideas. They maintained that they had seen smallpox victims who claimed to have had earlier cases of cowpox.

It became Jenner's task to transform a country superstition into an accepted medical practice. For up until the mid – 1770s, the only documented cases of vaccinations using cowpox came from farmers such as Benjamin Jesty of Dorsetshire who vaccinated his family with cowpox using a darning needle.

After observing cases of cowpox and smallpox for a quarter century, Jenner took a step that could have branded him a criminal as easily as a hero. On May 14, 1796, he removed the fluid from a cowpox lesion from dairymaid Sarah Nelmes, and inoculated James Phipps, an eight-year-old boy, who soon came down with cowpox. Six weeks later, he inoculated the boy with smallpox. The boy remained healthy. Jenner had proved his theory. He called his method vaccination, using the Latin word *vacca,* meaning cow, and *vaccinia,* meaning cowpox. He also introduced the word virus.

The publication of Jenner's *An Inquiry into the Causes and Effects of the Variolae Vaccinae* set off an enthusiastic demand for vaccination throughout Europe. Within 18 months, the number of deaths from smallpox had dropped by two-thirds in England after 12,000 people were vaccinated. By 1800, over 100,000 people had been vaccinated worldwide. As the demand for the **vaccine** rapidly increased, Jenner discovered that he could take lymph from a smallpox pustule and dry it in a glass tube for use up to three months later. The vaccine could then be transported.

Jenner was honored and respected throughout Europe and the United States. At his request, Napoleon released several Englishmen who had been jailed in France in 1804, while France and Great Britain were at war. Across the Atlantic Ocean, Thomas Jefferson received the vaccine from Jenner and proceeded to vaccinate his family and neighbors at Monticello. However, in his native England, Jenner's medical colleagues refused to allow him entry into the College of

Physicians in London, insisting that he first pass a test on the theories of Hippocrates and Galen. Jenner refused to bow to their demands, saying his accomplishments in conquering smallpox should have qualified him for election. He was never elected to the college. Jenner continued his medical practice, as well as collecting fossils and propagating hybrid plants in his garden, until his death from a stroke at the age of 73.

Nearly two centuries after Jenner's experimental vaccination of young James, the **World Health Organization** declared endemic smallpox to be eradicated.

See also Antibody and antigen; Antibody formation and kinetics; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation

JERNE, NIELS K. (1911-1994)

Danish immunologist

Often considered the founder of modern cellular **immunology**, Niels K. Jerne shared the 1984 Nobel Prize for medicine or physiology with **César Milstein** and Georges J. F. Köhler for his body of work that explained the function of the **immune system**, the body's defense mechanism against disease and infection. He is best known for three theories showing how antibodies—the substances which protect the body from foreign substances such as **viruses** and poisons—are produced, formed, and regulated by the immune system. His theories were initially met with skepticism, but they later became the cornerstones of immunological knowledge. By 1984, when Jerne received the prize, colleagues agreed that he should have been recognized for his important contributions to the field much earlier than he was. Jerne's theories became the starting point from which other scientists, notably 1960 Nobel Prize winner Frank MacFarlane Burnett, furthered understanding of how the body protects itself against disease.

Niels Kaj (sometimes translated Kai) Jerne was born in London, England, to Danish parents Else Marie Lindberg and Hans Jessen Jerne. The family moved to the Netherlands at the beginning of World War I. Jerne earned his baccalaureate in Rotterdam in 1928, and studied physics for two years at the University of Leiden. Twelve years later, he entered the University of Copenhagen to study medicine, receiving his doctorate in 1951 at the age of forty. From 1943 until 1956 he worked at the Danish State Serum Institute, conducting research in immunology.

In 1955, Jerne traveled to the United States with noted molecular biologist Max Delbrück to become a research fellow at the California Institute of Technology at Pasadena. The two worked closely together, and it was not until his final two weeks at the institute that Jerne completed work on his first major theory—on selective **antibody formation**. At this time, scientists accepted that specific antibodies do not exist until an antigen—any substance originating outside the body (e.g., a virus, snake venom, transplanted organs, etc.)—is introduced, and acts as a template from which cells in the immune system create the appropriate **antibody** to eliminate it. Antigens and antibodies have surface patches, called combining sites, with distinct patterns. When an **antibody and antigen** with complementary combining sites meet, they become attached, fitting together like a lock and key. Jerne's theory postulated instead that the immune system inherently contains all the specific antibodies it needs to fight specific antigens. The appropriate antibody, one of millions that are already present in the body, attaches to the **antigen**, thus neutralizing or destroying the antigen and its threat to the body.

Not until some months after developing his theory did Jerne shared it with Delbrück, who sent it to the *Proceedings of the National Academy of Sciences* for publication. Jerne later noted that his theory probably would have been forgotten, except that it caught the attention of Burnett, leading him to the development in 1959 of his clonal **selection** theory, which built on Jerne's hypothesis to show how specific antibody-producing cells multiply to produce necessary quantities of an antigen's antibody. The following year, Jerne left his research in immunology to become chief medical officer with the **World Health Organization** in Geneva, Switzerland, where he oversaw the departments of biological standards and immunology. From 1960 to 1962, he served on the faculty at the University of Geneva's biophysics department.

From 1962 to 1966, Jerne was professor of microbiology at the University of Pittsburgh in Pennsylvania. During this period, he developed a method, now known as the Jerne **plaque** assay, to count antibody-producing cells by first mixing them with other cells containing antigen material, causing the cells to produce an antibody that combines with red blood cells. Once combined, the blood cells are then destroyed, leaving a substance called plaque surrounding the original antibody-producing cells, which can then be counted. Jerne became director of the **Paul Ehrlich** Institute, in Frankfurt, Germany, in 1966, and, in 1969, established the Basel Institute for Immunology in Switzerland, where he remained until taking emeritus status in 1980.

In 1971, Jerne unveiled his second major theory, which deals with how the immune system identifies and differentiates between self molecules (belonging to its host) and nonself molecules (invaders). Noting that the immune system is specific to each individual, immunologists had concluded that the body's self-tolerance cannot be inherited, and is therefore learned. Jerne postulated that such immune system "learning" occurs in the thymus, an organ in the upper chest cavity where the cells that recognize and attack antigens multiply, while those that could attack the body's own cells are suppressed. Over time, **mutations** among cells that recognize antigens increase the number of different antibodies the body has at hand, thereby increasing the immune system's arsenal against disease.

Jerne introduced what is considered his most significant work in 1974—the network theory, wherein he proposed that the immune system is a dynamic self-regulating network that activates itself when necessary and shuts down when not needed. At that time, scientists knew that the immune system contains two types of immune system cells, or lymphocytes: **B cells**, which produce antibodies, and **T cells**, which function as "helpers" to the B cells by killing foreign cells, or by regulating the B cells either by suppressing or stimulating their antibody producing activity. Further, antibody molecules

produced by the B cells also contain antigen-like components that can attract another antibody (anti-idiotype), allowing one antibody to recognize another antibody as well as an antigen. Jerne's theory expanded on this knowledge, speculating that a delicate balance of lymphocytes and antibodies and their idiotypes and anti-idiotypes exists in the immune system until an antigen is introduced. The antigen, he argued, replaces the anti-idiotype attached to the antibody. The immune system then senses the displacement and, in an attempt to find the anti-idiotype a "mate," produces more of the original antibody. This chain-reaction strengthens the body's **immunity** to the invading antigen. Experiments later demonstrated that **immunization** with an anti-idiotype would stimulate the production of the required antibody. It may well be that because of Jerne's network theory, vaccinations of the future will administer antibodies rather than antigens to bring about immunity to disease.

Jerne retired to southern France with his wife. A citizen of both Denmark and Great Britain, Jerne received honorary degrees from American and European universities, was a foreign honorary member of the American Academy of Arts and Sciences, a member of the Royal Danish Academy of Sciences, and won, among other honors, the Marcel Benorst Prize in 1979, and the Paul Ehrlich Prize in 1982.

See also B cells or B lymphocytes; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunochemistry; T cells or T lymphocytes

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KELP AND KELP FORESTS

Brown algae, also known as kelps, are a group of seaweeds in the order Phaeophyta. They attach to rocks on the sea bottom by a tissue known as their holdfast, from which their flexible stems (known as a stipe) and leaf-like tissue (or fronds) grow into the water column. In some species, the fronds are kept buoyant by gas-filled bladders. Kelp tissues are extremely tough only the strongest storms are capable of tearing their fronds or ripping their holdfasts from the rocky bottom. When this happens, however, large masses of kelp biomass can float around as debris known as "paddies," or wash onto the shore as "wrack."

In some temperate marine habitats, large species of brown algae can be extremely abundant. These ecosystems are known as kelp forests. Because they are extremely productive ecosystems, and have a great deal of physical structure associated with their seaweed biomass, kelp forests provide habitat for a wide range of marine organisms. These include a diversity of species of smaller algae, invertebrates, fish, marine mammals, and birds. The kelp forests of the Pacific coast of North America are estimated to support more than 1,000 species of marine plants and animals.

Kelp forests occur in many parts of the world, including the Atlantic and Pacific coasts of North America. However, the tallest, best-developed kelp forests are in waters 20–210 ft (6–70 m) deep over rocky bottoms off the coast of California. This ecosystem is dominated by the giant kelp (*Macrocystis pyrifera*), which ranges from central California to Baja California (the genus also occurs on the west coast of South America, and off South Africa, southern Australia, and New Zealand). This enormous seaweed is also known as the giant bladder kelp because of the flotation structures attached to its fronds. The giant kelp begins its life as a microscopic spore, but can grow as immensely long as 200 ft (60 m) and live for 4–7 years. Most of its photosynthetic activity occurs in the upper part of its tall canopy, because the lower areas are intensely shaded and do not receive much sunlight.

Other, somewhat smaller species of *Macrocystis* occur more widely along the Pacific coast, as far north as southern Alaska. Other giant seaweeds of kelp forests of the Pacific coast include the bull kelp (*Nereocystis leutkeana*), the elk horn kelp (*Pelagophycus porra*), the feather boa kelp (*Egregia menziesii*), and the Fucalean alga (*Cystoseira osmundacea*).

Sea urchins are marine invertebrates that feed voraciously on kelp biomass (they are herbivores meaning that plants are their primary source of food). Periodically, sea urchins of the genus *Strongylocentrotus* can become extremely abundant and cause an intense disturbance to the kelp-forest ecosystem. They do this by feeding on the holdfasts and causing the kelp to detach from their rocky anchors, resulting in an ecosystem known as an "urchin barren" because it sustains so little biomass of seaweeds or other species. This sort of natural ecological damage has been observed numerous times, in various parts of the world.

Off the coast of western North America, however, sea otters (*Enhydra lutris*) feed on the urchins and can prevent them from becoming too abundant, thereby keeping the kelp forests intact. This ecological balance among sea urchins, sea otters, and kelps became upset during the nineteenth century, when the populations of the otters were virtually wiped out by excessive hunting for the fur trade. Because of the collapse of otter populations, the urchins became more abundant. Their excessive feeding on kelps greatly reduced the extent and luxuriance of the kelp forests. Fortunately, this balance has since been restored by the cessation of the hunting of sea otters, allowing them to again control the abundance of the urchins. In turn, the productive kelp forests have been able to redevelop.

Seaweed biomass contains a number of useful chemicals, such as alginates used as thickeners and gelling agents in a wide variety of manufactured products. A minor use is as a food supplement. In some regions, kelps are being harvested as an economic resource to supply these industrial chemicals. Off the coast of California, for example, kelp harvesting amounts to as much as 176,000 tons (160,000 metric tons) per year. If the harvesting method takes care to not damage the

holdfasts and other deeper tissues of the kelps, then the forest can regenerate quite well from the disturbance. In California, for example, kelp harvesters are only allowed to cut in the top 4 ft (1.4 m) of the water column, leaving the deeper parts of the forest intact. The kelp harvesting is done using a large barge-like apparatus, which can collect as much as 605 tons (550 metric tons) of kelp per day.

Kelp forests also have an extremely large indirect value to the economy, by serving as the critical habitat for many species of fish and shellfish that are harvested in the coastal fishery. The forests are also critical habitat for many species of indigenous biodiversity. This has an indirect benefit to the coastal economy, through recreational activities associated with ecotourism.

KHORANA, HAR GOBIND (1922-)

Indian-born American biochemist

Har Gobind Khorana, an organic chemist who specialized in the study of proteins and nucleic acids, shared the Nobel Prize in Physiology of Medicine with Robert W. Holley (1922–) and Marshall W. Nirenberg (1927–) in 1968 for discoveries related to the **genetic code** and its function in **protein synthesis**. In addition to developing methods for investigating the structure of the nucleic acids, Khorana introduced many of the techniques that allowed scientists to decipher the genetic code and show how **ribonucleic acid** (**RNA**) can specify the structure of proteins. Four years after winning the Nobel Prize, Khorana succeeded in synthesizing the first wholly artificial **gene**. In the 1980s Khorana synthesized the gene for rhodopsin, a protein involved in vision.

Har Gobind Khorana, youngest of the five children of Shri Ganput Rai Khorana and Shrimat Krishna Devi Khorana, was born in Raipur, in the Punjab region of India (now part of West Pakistan). His birth date was recorded as January 9, 1922, but the exact date of his birth is uncertain. Although his family was poor, his parents believed strongly in the importance of education. His father was a village agricultural taxation clerk in the British colonial government. Khorana attended D.A.V. High School in Multan (now West Punjab). After receiving his Bachelor of Science (1943, with honors) and Master's degree (1945, with honors) from Punjab University in Lahore, India, Khorana was awarded a Government of India Fellowship, which enabled him to study at Liverpool University, England, where he earned his Ph.D. in 1948. From 1948 to 1949, he worked as a postdoctoral fellow at the Federal Institute of Technology, Zurich, Switzerland, with Professor Vladimir Prelog, who had a major influence on his life-long approach to science.

After briefly returning to India, Khorana accepted a position in the laboratory of (Lord) Alexander Todd at Cambridge University (1950–52), where he studied proteins and nucleic acids. From 1952 to 1960, Khorana worked in the organic chemistry section of the British Columbia Research Council, Vancouver, Canada. The next year Khorana moved to the University of Wisconsin, Madison, Wisconsin, where he served as Co-director of the Institute for Enzyme Research and Professor of **Biochemistry**. In 1964, he became the Conrad A. Elvehjem Professor of the Life Sciences. In 1970, Khorana accepted the position of Alfred P. Sloan Professor, Departments of Biology and Chemistry, at the Massachusetts Institute of Technology, Cambridge, Massachusetts. From 1974 to 1980, he was Andrew D. White Professor-at-large, Cornell University, Ithaca, New York. During his long and distinguished career, Khorana has been the author or co-author of over 500 scientific publications.

In 1953, Khorana and Todd published their only co-authored paper; it described the use of a novel phosphorylating reagent. Khorana found that this reagent was very useful in overcoming problems in the synthesis of polynucleotides. Between 1956 and 1958, Khorana and his coworkers established the fundamental techniques of nucleotide chemistry. Their goal was to develop purely chemical methods of synthesizing oligonucleotides (long chains of nucleotides). In 1961, Khorana synthesized Coenzyme A, a factor needed for the activity of certain key metabolic **enzymes**.

In 1955, Khorana learned about Severo Ochoa's discovery of the enzyme polynucleotide phosphorylase and met Arthur Kornberg, who described pioneering research on the enzymatic synthesis of **DNA**. These discoveries revolutionized nucleic acid research and made it possible to elucidate the genetic code. Khorana and his coworkers synthesized each of the 64 possible triplets (codons) by synthesizing polynucleotides of known composition. Khorana also devised the methods that led to the synthesis of large, well-defined nucleic acids.

By combining synthetic and enzymatic methods, Khorana was able to overcome many obstacles to the chemical synthesis of polyribonucleotides. Khorana's work provided unequivocal proof of codon assignments and defined some codons that had not been determined by other methods. Some triplets, which did not seem to code for any particular amino acid, were shown to serve as "punctuation marks" for beginning and ending the synthesis of polypeptide chains (long chains of amino acids). Khorana's investigations also provided direct evidence concerning other characteristics of the genetic code. For example, Khorana's work proved that three nucleotides specify an amino acid, provided proof of the direction in which the information in messenger RNA is read, demonstrated that punctuation between codons is unnecessary, and that the codons did not overlap. Moreover, construction of specific polyribonucleotides proved that an RNA intermediary is involved in translating the sequence of nucleotides in DNA into the sequence of amino acids in a protein. Summarizing the remarkable progress that had been made up to 1968 in polynucleotide synthesis and understanding the genetic code, Khorana remarked that the nature of the genetic code was fairly well established, at least for *Escherichia coli*.

Once the genetic code had been elucidated, Khorana focused on gene structure-gene function relationships and studies of DNA-protein interactions. In order to understand gene expression, Khorana turned to DNA synthesis and sequencing. Recognizing the importance of the class of ribonucleotides known as transfer RNAs (tRNAs), Khorana decided to synthesize the DNA sequence that coded for ala-

nine tRNA. The nucleotide sequence of this tRNA had been determined in Robert Holley's laboratory. In 1970, when Khorana announced the total synthesis of the first wholly artificial gene, his achievement was honored as a major landmark in **molecular biology**. Six years later, Khorana and his associates synthesized another gene. In the 1980s, Khorana carried out studies of the chemistry and molecular biology of the gene for rhodopsin, a protein involved in vision.

In 1966, Khorana was elected to the National Academy of Sciences. His many honors and awards include the Merck Award from the Chemical Institute of Canada, the Dannie-Heinneman Prize, the American Chemical Society Award for Creative Work in Synthetic Organic Chemistry, the Lasker Foundation Award for Basic Medical Research, the Padma Vibhushan Presidential Award, the Ellis Island Medal of Honor, the National Medal of Science, and the Paul Kayser International Award of Merit in Retina Research. He holds Honorary Degrees for numerous universities, including Simon Fraser University, Vancouver, Canada; University of Liverpool, England; University of Punjab, India; University of Delhi, India; Calcutta University, India; University of Chicago; and University of British Columbia, Vancouver, Canada.

See also Genetic regulation of eukaryotic cells; Microbial genetics

KITASATO, SHIBASABURO (1852-1931)

Japanese bacteriologist

Bacteriologist Shibasaburo Kitasato made several important contributions to the understanding of human disease and how the body fights off infection. He also discovered the bacterium that causes **bubonic plague**.

Born in Kumamoto, Japan, Kitasato, completed his medical studies at the University of Tokyo in 1883. Shortly after, he traveled to Berlin to work in the laboratory of **Robert Koch**. Among his greatest accomplishments, Kitasato discovered a way of growing a pure **culture** of **tetanus** bacillus using anaerobic methods in 1889. In the following year, Kitasato and German microbiologist **Emil von Behring** reported on the discovery of tetanus and **diphtheria** antitoxin. They found that animals injected with the microbes that cause tetanus or diphtheria produced substances in their blood, called antitoxins, which neutralized the toxins produced by the microbes. Furthermore, these antitoxins could be injected into healthy animals, providing them with **immunity** to the microbes. This was a major finding in explaining the workings of the **immune system**. Kitasato went on to discover **anthrax** antitoxin as well.

In 1892, Kitasato returned to Tokyo and founded his own laboratory. Seven years later, the laboratory was taken over by the Japanese government, and Kitasato was appointed its director. When the laboratory was consolidated with the University of Tokyo, however, Kitasato resigned and founded the Kitasato Institute.

During an outbreak of the bubonic plague in Hong Kong in 1894, Kitasato was sent by the Japanese government to research the disease. He isolated the bacterium that caused the plague. (Alexandre Yersin, 1863 – 1943, independently announced the discovery of the organism at the same time). Four years later, Kitasato and his student Kigoshi Shiga were able to isolate and describe the organism that caused one form of **dysentery**.

Kitasato was named the first president of the Japanese Medical Association in 1923, and was made a baron by the Emperor in 1924. He died in Japan in 1931.

See also Antibody and antigen; Bacteria and bacterial infection; Immunity, active, passive and delayed; Immunization

KLUYVER, ALBERT JAN (1888-1956)

Dutch microbiologist, biochemist, and botanist

Albert Jan Kluyver developed the first general model of cell **metabolism** in both aerobic and anaerobic **microorganisms**, based on the transfer of hydrogen atoms. He was a major exponent of the "Delft School" of classical microbiology in the tradition of Antoni van Leeuwenhoek (1632–1723). Outside Delft, he also drew on the legacy of **Louis Pasteur** (1822–1895), **Robert Koch** (1843–1910), and Sergei Nikolayevich Winogradsky (1856–1953).

Born in Breda, the Netherlands, on June 3, 1888, Kluyver was the son of a mathematician and engineer, Jan Cornelis Kluyver, and his wife, Marie, née Honingh. In 1910, he received his bachelor's degree in chemical engineering from the Delft University of Technology, but immediately shifted his focus toward botany and **biochemistry**, winning his doctorate in 1914 with a dissertation on the determinations of biochemical sugars under the tutelage of Gijsebertus van Iterson, professor of microscopic anatomy. In 1916, on van Iterson's recommendation, the Dutch government appointed Kluyver as an agricultural and biological consultant for the Dutch East Indies colonial administration.

In 1921, again on van Iterson's recommendation, Kluyver succeeded **Martinus Willem Beijerinck** (1851–1931) as director of the microbiology laboratory at Delft, where he spent the rest of his career. He immediately acquired the most modern equipment and established high standards for both collegiality and research. The reorganized laboratory thrived. Kluyver's reputation soon attracted many excellent graduate students, such as Cornelius Bernardus van Niel (1897–1985), another chemical engineer. Van Niel received his doctorate under Kluyver with a dissertation on propionic acid **bacteria** in 1928 and was immediately offered an appointment at Stanford University.

In a landmark paper, "Eenheid en verscheidenheid in de stofwisseling der microben" [Unity and diversity in the metabolism of microorganisms] *Chemische Weekblad*, Kluyver examined the metabolic processes of oxidation and **fermentation** to conclude that, without bacteria and other microbes, all life would be impossible. Two years later he co-authored with his assistant, Hendrick Jean Louis Donker, another important paper, "Die Einheit in der Biochemie" [Unity in biochemistry] *Chemie der Zelle und Gewebe*, which asserted that all life forms are chemically interdependent because of their shared

and symbiotic metabolic needs. He explained these findings further in *The Chemical Activities of Microorganisms*.

Kluyver had a knack for bringing out the best in his students. He often and fruitfully collaborated and co-published with them, maintaining professional relationships with them long after they left Delft. For example, with van Niel he co-wrote *The Microbe's Contribution to Biology*. A cheerful, friendly, popular man, he was widely and fondly eulogized when he died in Delft on May 14, 1956. Van Niel called him "The Father of Comparative Biochemistry."

See also Aerobes; Anaerobes and anaerobic infections; Azotobacter; Bacteria and bacterial infection; Bioluminescence; *Escherichia coli (E.coli)*; Microbial symbiosis; Microbial taxonomy; Microscope and microscopy; Yeast

KOCH, ROBERT (1843-1910)

German physician

Robert Koch pioneered principles and techniques in studying **bacteria** and discovered the specific agents that cause **tuberculosis**, cholera, and **anthrax**. For this he is often regarded as a founder of microbiology and **public health**, aiding legislation and changing prevailing attitudes about **hygiene** to prevent the spread of various infectious diseases. For his work on tuberculosis, he was awarded the Nobel Prize in 1905.

Robert Heinrich Hermann Koch was born in a small town near Klausthal, Hanover, Germany, to Hermann Koch, an administrator in the local mines, and Mathilde Julie Henriette Biewend, a daughter of a mine inspector. The Kochs had thirteen children, two of whom died in infancy. Robert was the third son. Both parents were industrious and ambitious. Robert's father rose in the ranks of the mining industry, becoming the overseer of all the local mines. His mother passed her love of nature on to Robert who, at an early age, collected various plants and insects.

Before starting primary school in 1848, Robert taught himself to read and write. At the top of his class during his early school years, he had to repeat his final year. Nevertheless, he graduated in 1862 with good marks in the sciences and mathematics. A university education became available to Robert when his father was once again promoted and the family's finances improved. Robert decided to study natural sciences at Göttingen University, close to his home.

After two semesters, Koch transferred his field of study to medicine. He had dreams of becoming a physician on a ship. His father had traveled widely in Europe and passed a desire for travel on to his son. Although bacteriology was not taught then at the University, Koch would later credit his interest in that field to Jacob Henle, an anatomist who had published a theory of contagion in 1840. Many ideas about contagious diseases, particularly those of chemist and microbiologist **Louis Pasteur**, who was challenging the prevailing myth of spontaneous generation, were still being debated in universities in the 1860s.

During Koch's fifth semester at medical school, Henle recruited him to participate in a research project on the structure of uterine nerves. The resulting essay won first prize. It was dedicated to his father and bore the Latin motto, *Nunquam Otiosus,*, meaning never idle. During his sixth semester, he assisted Georg Meissner at the Physiological Institute. There he studied the secretion of succinic acid in animals fed only on fat. Koch decided to experiment on himself, eating a half-pound of butter each day. After five days, however, he was so sick that he limited his study to animals. The findings of this study eventually became Koch's dissertation. In January 1866, he finished the final exams for medical school and graduated with highest distinction.

After finishing medical school, Koch held various positions; he worked as an assistant at a hospital in Hamburg, where he became familiar with cholera, and also as an assistant at a hospital for developmentally delayed children. In addition, he made several attempts to establish a private practice. In July, 1867, he married Emmy Adolfine Josephine Fraatz, a daughter of an official in his hometown. Their only child, a daughter, was born in 1868. Koch finally succeeded in establishing a practice in the small town of Rakwitz where he settled with his family.

Shortly after moving to Rakwitz, the Franco-Prussian War broke out and Koch volunteered as a field hospital physician. In 1871, the citizens of Rakwitz petitioned Koch to return to their town. He responded, leaving the army to resume his practice, but he didn't stay long. He soon took the exams to qualify for district medical officer and in August 1872 was appointed to a vacant position at Wollstein, a small town near the Polish border.

It was here that Koch's ambitions were finally able to flourish. Though he continued to see patients, Koch converted part of his office into a laboratory. He obtained a **microscope** and observed, at close range, the diseases his patients confronted him with.

One such disease was anthrax, which is spread from animals to humans through contaminated wool, by eating uncooked meat, or by breathing in airborne spores emanating from contaminated products. Koch examined under the microscope the blood of infected sheep and saw specific **microorganisms** that confirmed a thesis put forth ten years earlier by biologist C. J. Davaine that anthrax was caused by a bacillus. Koch attempted to **culture** (grow) these bacilli in cattle blood so he could observe their life cycle, including their formation into spores and their germination. Koch performed scrupulous research both in the laboratory and in animals before showing his work to Ferdinand Cohn, a botanist at the University of Breslau. Cohn was impressed with the work and replicated the findings in his own laboratory. He published Koch's paper in 1876.

In 1877, Koch published another paper that elucidated the techniques he had used to isolate *Bacillus anthracis*. He had dry-fixed bacterial cultures onto glass slides, then stained the cultures with dyes to better observe them, and photographed them through the microscope.

It was only a matter of time that Koch's research eclipsed his practice. In 1880, he accepted an appointment as a government advisor with the Imperial Department of Health in Berlin. His task was to develop methods of isolating and

cultivating disease-producing bacteria and to formulate strategies for preventing their spread. In 1881 he published a report advocating the importance of pure cultures in isolating disease-causing organisms and describing in detail how to obtain them. The methods and theory espoused in this paper are still considered fundamental to the field of modern bacteriology. Four basic criteria, now known as **Koch's postulates**, are essential for an organism to be identified as pathogenic, or capable of causing disease. First, the organism must be found in the tissues of animals with the disease and not in disease-free animals. Second, the organism must be isolated from the diseased animal and grown in a pure culture outside the body, or *in vitro*. Third, the cultured organism must be able to be transferred to a healthy animal, which will subsequently show signs of infection. And fourth, the organisms must be able to be isolated from the infected animal.

While in Berlin, Koch became interested in tuberculosis, which he was convinced was infectious, and, therefore, caused by a bacterium. Several scientists had made similar claims but none had been verified. Many other scientists persisted in believing that tuberculosis was an inherited disease. In six months, Koch succeeded in isolating a bacillus from tissues of humans and animals infected with tuberculosis. In 1882, he published a paper declaring that this bacillus met his four conditions—that is, it was isolated from diseased animals, it was grown in a pure culture, it was transferred to a healthy animal who then developed the disease, and it was isolated from the animal infected by the cultured organism. When he presented his findings before the Physiological Society in Berlin on March 24, he held the audience spellbound, so logical and thorough was his delivery of this important finding. This day has come to be known as the day modern bacteriology was born.

In 1883, Koch's work on tuberculosis was interrupted by the Hygiene Exhibition in Berlin, which, as part of his duties with the health department, he helped organize. Later that year, he finally realized his dreams of travel when he was invited to head a delegation to Egypt where an outbreak of cholera had occurred. Louis Pasteur had hypothesized that cholera was caused by a microorganism; within three weeks, Koch had identified a comma-shaped organism in the intestines of people who had died of cholera. However, when testing this organism against his four postulates, he found that the disease did not spread when injected into other animals. Undeterred, Koch proceeded to India where cholera was also a growing problem. There, he succeeded in finding the same organism in the intestines of the victims of cholera, and although he was still unable to induce the disease in experimental animals, he did identify the bacillus when he examined, under the microscope, water from the ponds used for drinking water. He remained convinced that this bacillus was the cause of cholera and that the key to prevention lay in improving hygiene and sanitation.

Koch returned to Germany and from 1885–1890 was administrator and professor at Berlin University. He was highly praised for his work, though some high-ranking scientists and doctors continued to disagree with his conclusions. Koch was an adept researcher, able to support each claim with his exacting methodology. In 1890, however, Koch faltered

Robert Koch, whose postulates on the identification of microorganisms as the cause of a disease remain a fundamental underpinning of infectious microbiology.

from his usual perfectionism and announced at the International Medical Congress in Berlin that he had found an inoculum that could prevent tuberculosis. He called this agent tuberculin. People flocked to Berlin in hopes of a cure and Koch was persuaded to keep the exact formulation of tuberculin a secret, in order to discourage imitations. Although optimistic reports had come out of the clinical trials Koch had set up, it soon became clear from autopsies that tuberculin was causing severe **inflammation** in many patients. In January 1891, under pressure from other scientists, Koch finally published the nature of the substance, but it was an uncharacteristically vague and misleading report which came under immediate criticism from his peers.

Koch left Berlin for a time after this incident to recover from the professional setback, although the German government continued to support him throughout this time. An Institute for Infectious Diseases was established and Koch was named director. With a team of researchers, he continued his work with tuberculin, attempting to determine the ideal dose at which the agent could be the safest and most effective. The

discovery that tuberculin was a valuable diagnostic tool (causing a reaction in those infected but none in those not infected), rather than a cure, helped restore Koch's reputation. In 1892, there was a cholera outbreak in Hamburg. Thousands of people died. Koch advocated strict sanitary conditions and isolation of those found to be infected with the bacillus. Germany's senior hygienist, Max von Pettenkofer, was unconvinced that the bacillus alone could cause cholera. He doubted Koch's ideas, going so far as to drink a freshly isolated culture. Several of his colleagues joined him in this demonstration. Two developed symptoms of cholera, Pettenkofer suffered from diarrhea, but no one died; Pettenkofer felt vindicated in his opposition to Koch. Nevertheless, Koch focused much of his energy on testing the water supply of Hamburg and Berlin and perfecting techniques for filtering drinking water to prevent the spread of the bacillus.

In the following years, he gave the directorship of the Institute over to one of his students so he could travel again. He went to India, New Guinea, Africa, and Italy, where he studied diseases such as the plague, **malaria**, **rabies**, and various unexplained fevers. In 1905, after returning to Berlin from Africa, he was awarded the Nobel Prize for physiology and medicine for his work on tuberculosis. Subsequently, many other honors were awarded him recognizing not only his work on tuberculosis, but his more recent research on tropical diseases, including the Prussian Order Pour le Merits in 1906 and the Robert Koch medal in 1908. The Robert Koch Medal was established to honor the greatest living physicians, and the Robert Koch Foundation, established with generous grants from the German government and from the American philanthropist, Andrew Carnegie, was founded to work toward the eradication of tuberculosis.

Meanwhile, Koch settled back into the Institute where he supervised clinical trials and production of new tuberculins. He attempted to answer, once and for all, the question of whether tuberculosis in cattle was the same disease as it was in humans. Between 1882 and 1901 he had changed his mind on this question, coming to accept that bovine tuberculosis was not a danger to humans, as he had previously thought. He presented his arguments at conferences in the United States and Britain during a time when many governments were attempting large-scale efforts to minimize the transmission of tuberculosis through limiting meat and milk.

Koch did not live to see this question answered. On April 9, 1910, three days after lecturing on tuberculosis at the Berlin Academy of Sciences, he suffered a heart attack from which he never fully recovered. He died at Baden Baden the next month at the age of 67. He was honored after death by the naming of the Institute after him. In the first paper he wrote on tuberculosis, he stated his lifelong goal, which he clearly achieved: "I have undertaken my investigations in the interests of public health and I hope the greatest benefits will accrue therefrom."

See also Bacteria and bacterial infection; History of microbiology; History of public health; Koch's postulates; Laboratory techniques in microbiology

KOCH'S POSTULATES

Koch's postulates are a series of conditions that must be met for a microorganism to be considered the cause of a disease. German microbiologist **Robert Koch** (1843–1910) proposed the postulates in 1890.

Koch originally proposed the postulates in reference to bacterial diseases. However, with some qualifications, the postulates can be applied to diseases caused by **viruses** and other infectious agents as well.

According to the original postulates, there are four conditions that must be met for an organism to be the cause of a disease. Firstly, the organism must be present in every case of the disease. If not, the organism is a secondary cause of the infection, or is coincidentally present while having no active role in the infection. Secondly, the organism must be able to be isolated from the host and grown in the artificial and controlled conditions of the laboratory. Being able to obtain the microbe in a pure form is necessary for the third postulate that stipulates that the disease must be reproduced when the isolated organism is introduced into another, healthy host. The fourth postulate stipulates that the same organism must be able to be recovered and purified from the host that was experimentally infected.

Since the proposal and general acceptance of the postulates, they have proven to have a number of limitations. For example, infections organisms such as some the bacterium *Mycobacterium leprae*, some viruses, and **prions** cannot be grown in artificial laboratory media. Additionally, the postulates are fulfilled for a human disease-causing microorganism by using test animals. While a microorganism can be isolated from a human, the subsequent use of the organism to infect a healthy person is unethical. Fulfillment of Koch's postulates requires the use of an animal that mimics the human infection as closely as is possible.

Another limitation of Koch's postulates concerns instances where a microorganism that is normally part of the normal flora of a host becomes capable of causing disease when introduced into a different environment in the host (e.g., *Staphylococcus aureus*), or when the host's **immune system** is malfunctioning (e.g., *Serratia marcescens*.

Despite these limitations, Koch's postulates have been very useful in clarifying the relationship between **microorganisms** and disease.

See also Animal models of infection; Bacteria and bacterial infection; Germ theory of disease; History of immunology; History of microbiology; History of public health; Laboratory techniques in immunology; Laboratory techniques in microbiology

KÖHLER, GEORGES (1946-1995)

German immunologist

For decades, antibodies, substances manufactured by the plasma cells to help fight disease, were produced artificially by injecting animals with foreign macromolecules, then

extracted by bleeding the animals and separating the **antiserum** in their blood. The technique was arduous and far from foolproof. But the discovery of the hybridoma technique by German immunologist Georges Köhler changed revolutionize the procedure. Köhler's work made antibodies relatively easy to produce and dramatically facilitated research on many serious medical disorders such as acquired **immunodeficiency** syndrome (**AIDS**) and cancer. For his work on what would come to be known as monoclonal antibodies, Köhler shared the 1984 Nobel Prize in medicine.

Born in Munich, in what was then occupied Germany, Georges Jean Franz Köhler attended the University of Freiburg, where he obtained his Ph.D. in biology in 1974. From there he set off to Cambridge University in England, to work as a postdoctoral fellow for two years at the British Medical Research Council's laboratories. At Cambridge, Köhler worked under Dr. **César Milstein**, an Argentinean-born researcher with whom Köhler would eventually share the Nobel Prize. At the time, Milstein, who was Köhler's senior by nineteen years, was a distinguished immunologist, and he actively encouraged Köhler in his research interests. Eventually, it was while working in the Cambridge laboratory that Köhler discovered the hybridoma technique.

Dubbed by the *New York Times* as the "guided missiles of biology," antibodies are produced by human plasma cells in response to any threatening and harmful bacterium, virus, or tumor cell. The body forms a specific **antibody** against each **antigen**; and César Milstein once told the *New York Times* that the potential number of different antigens may reach "well over a million." Therefore, for researchers working to combat diseases like cancer, an understanding of how antibodies could be harnessed for a possible cure is of great interest. And although scientists knew the benefits of producing antibodies, until Köhler and Milstein published their findings, there was no known technique for maintaining the long-term **culture** of antibody-forming plasma cells.

Köhler's interest in the subject had been aroused years earlier, when he had become intrigued by the work of Dr. Michael Potterof the National Cancer Institute in Bethesda, Maryland. In 1962 Potter had induced myelomas, or plasma-cell tumors in mice, and others had discovered how to keep those tumors growing indefinitely in culture. Potter showed that plasma tumor cells were both seemingly immortal and able to create an unlimited number of identical antibodies. The only drawback was that there seemed no way to make the cells produce a certain *type* of antibody. Because of this, Köhler wanted to initiate a **cloning** experiment that would fuse plasma cells able to produce the desired antibodies with the "immortal" myeloma cells. With Milstein's blessing, Köhler began his experiment.

"For seven weeks after he had made the hybrid cells," the *New York Times* reported in October, 1984, "Dr. Köhler refrained from testing the outcome of the experiment for fear of likely disappointment. At last, around Christmas 1974, he persuaded his wife," Claudia Köhler, "to come to the windowless basement where he worked to share his anticipated disappointment after the critical test." But disappointment turned to joy when Köhler discovered his test had been a success: Astoundingly, his hybrid cells were making pure antibodies against the test antigen. The result was dubbed monoclonal antibodies. For his contribution to medical science, Köhler—who in 1977 had relocated to Switzerland to do research at the Basel Institute for Immunology—was awarded the Nobel in 1984.

The implications of Köhler's discovery were immense, and opened new avenues of basic research. In the early 1980s Köhler's discovery led scientists to identify various lymphocytes, or white blood cells. Among the kinds discovered were the T-4 lymphocytes, the cells destroyed by AIDS. Monoclonal antibodies have also improved tests for **hepatitis** B and streptococcal infections by providing guidance in selecting appropriate **antibiotics**, and they have aided in the research on thyroid disorders, lupus, rheumatoid arthritis, and inherited brain disorders. More significantly, Köhler's work has led to advances in research that can harness monoclonal antibodies into certain drugs and toxins that fight cancer, but would cause damage in their own right. Researchers are also using monoclonal antibodies to identify antigens specific to the surface of cancer cells so as to develop tests to detect the spread of cancerous cells in the body.

Despite the significance of the discovery, which has also resulted in vast amounts of research funds for many research laboratories, for Köhler and Milstein—who never patented their discovery—there was little financial remuneration. Following the award, however, he and Milstein, together with Michael Potter, were named winners of the Lasker Medical Research Award.

In 1985, Köhler moved back to his hometown of Freiburg, Germany, to assume the directorship of the Max Planck Institute for Immune Biology. He died in Freiburg in 1995.

See also Antibody-antigen, biochemical and molecular reactions; Antibody and antigen; Antibody formation and kinetics; Antibody, monoclonal; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunodeficiency; Immunodeficiency disease syndromes; Immunodeficiency diseases

KREBS, HANS ADOLF (1900-1981)

German biochemist

Few students complete an introductory biology course without learning about the **Krebs cycle**, an indispensable step in the process the body performs to convert food into energy. Also known as the citric acid cycle or tricarboxylic acid cycle, the Krebs cycle derives its name from one of the most influential biochemists of our time. Born in the same year as the twentieth century, Hans Adolf Krebs spent the greater part of his eighty-one years engaged in research on intermediary **metabolism**. First rising to scientific prominence for his work on the ornithine cycle of urea synthesis, Krebs shared the Nobel Prize for physiology and medicine in 1953 for his discovery of the citric acid cycle. Over the course of his career, the German-born scientist published, oversaw, or supervised a total of

more than 350 scientific publications. But the story of Krebs's life is more than a tally of scientific achievements; his biography can be seen as emblematic of biochemistry's path to recognition as its own discipline.

In 1900, Alma Davidson Krebs gave birth to her second child, a boy named Hans Adolf. The Krebs family—Hans, his parents, sister Elisabeth and brother Wolfgang—lived in Hildesheim, in Hanover, Germany. There his father Georg practiced medicine, specializing in surgery and diseases of the ear, nose, and throat. Hans developed a reputation as a loner at an early age. He enjoyed swimming, boating, and bicycling, but never excelled at athletic competitions. He also studied piano diligently, remaining close to his teacher throughout his university years. At the age of fifteen, the young Krebs decided he wanted to follow in his father's footsteps and become a physician. World War I had broken out, however, and before he could begin his medical studies, he was drafted into the army upon turning eighteen in August of 1918. The following month he reported for service in a signal corps regiment in Hanover. He expected to serve for at least a year, but shortly after he started basic training, the war ended. Krebs received a discharge from the army to commence his studies as soon as possible.

Krebs chose the University of Göttingen, located near his parents' home. There, he enrolled in the basic science curriculum necessary for a student planning a medical career and studied anatomy, histology, embryology and botanical science. After a year at Göttingen, Krebs transferred to the University of Freiburg. At Freiburg, Krebs encountered two faculty members who enticed him further into the world of academic research: Franz Knoop, who lectured on physiological chemistry, and Wilhelm von Möllendorff, who worked on histological staining. Möllendorff gave Krebs his first research project, a comparative study of the staining effects of different dyes on muscle tissues. Impressed with Krebs's insight that the efficacy of the different dyes stemmed from how dispersed and dense they were rather than from their chemical properties, Möllendorff helped Krebs write and publish his first scientific paper. In 1921, Krebs switched universities again, transferring to the University of Munich, where he started clinical work under the tutelage of two renowned surgeons. In 1923, he completed his medical examinations with an overall mark of "very good," the best score possible. Inspired by his university studies, Krebs decided against joining his father's practice as he had once planned; instead, he planned to balance a clinical career in medicine with experimental work. But before he could turn his attention to research, he had one more hurdle to complete, a required clinical year, which he served at the Third Medical Clinic of the University of Berlin.

Krebs spent his free time at the Third Medical Clinic engaged in scientific investigations connected to his clinical duties. At the hospital, Krebs met Annelise Wittgenstein, a more experienced clinician. The two began investigating physical and chemical factors that played substantial roles in the distribution of substances between blood, tissue, and cerebrospinal fluid, research that they hoped might shed some light on how pharmaceuticals such as those used in the treatment of **syphilis** penetrate the nervous system. Although Krebs published three articles on this work, later in life he belittled these early, independent efforts. His year in Berlin convinced Krebs that better knowledge of research chemistry was essential to medical practice.

Accordingly, the twenty-five-year-old Krebs enrolled in a course offered by Berlin's Charité Hospital for doctors who wanted additional training in laboratory chemistry. One year later, through a mutual acquaintance, he was offered a paid research assistantship by Otto Warburg, one of the leading biochemists of the time. Although many others who worked with Warburg called him autocratic, under his tutelage Krebs developed many habits that would stand him in good stead as his own research progressed. Six days a week work began at Warburg's laboratory at eight in the morning and concluded at six in the evening, with only a brief break for lunch. Warburg worked as hard as the students. Describing his mentor in his autobiography, *Hans Krebs: Reminiscences and Reflections,* Krebs noted that Warburg worked in his laboratory until eight days before he died from a pulmonary embolism. At the end of his career, Krebs wrote a biography of his teacher, the subtitle of which described his perception of Warburg: "cell physiologist, biochemist, and eccentric."

Krebs's first job in Warburg's laboratory entailed familiarizing himself with the tissue slice and manometric (pressure measurement) techniques the older scientist had developed. Until that time, biochemists had attempted to track chemical processes in whole organs, invariably experiencing difficulties controlling experimental conditions. Warburg's new technique, affording greater control, employed single layers of tissue suspended in solution and manometers (pressure gauges) to measure chemical reactions. In Warburg's lab, the tissue slice/manometric method was primarily used to measure rates of **respiration** and glycolysis, processes by which an organism delivers oxygen to tissue and converts carbohydrates to energy. Just as he did with all his assistants, Warburg assigned Krebs a problem related to his own research—the role of heavy metals in the oxidation of sugar. Once Krebs completed that project, he began researching the metabolism of human cancer tissue, again at Warburg's suggestion. While Warburg was jealous of his researchers' laboratory time, he was not stingy with bylines; during Krebs's four years in Warburg's lab, he amassed sixteen published papers. Warburg had no room in his lab for a scientist interested in pursuing his own research. When Krebs proposed undertaking studies of intermediary metabolism that had little relevance for Warburg's work, the supervisor suggested Krebs switch jobs.

Unfortunately for Krebs, the year was 1930. Times were hard in Germany, and research opportunities were few. He accepted a mainly clinical position at the Altona Municipal Hospital, which supported him while he searched for a more research-oriented post. Within the year, he moved back to Freiburg, where he worked as an assistant to an expert on metabolic diseases with both clinical and research duties. In the well-equipped Freiburg laboratory, Krebs began to test whether the tissue slice technique and manometry he had mastered in Warburg's lab could shed light on complex synthetic metabolic processes. Improving on the master's methods, he began using saline solutions in which the concentrations of

various ions matched their concentrations within the body, a technique which eventually was adopted in almost all biochemical, physiological, and pharmacological studies.

Working with a medical student named Kurt Henseleit, Krebs systematically investigated which substances most influenced the rate at which urea—the main solid component of mammalian urine—forms in liver slices. Krebs noticed that the rate of urea synthesis increased dramatically in the presence of ornithine, an amino acid present during urine production. Inverting the reaction, he speculated that the same ornithine produced in this synthesis underwent a cycle of conversion and synthesis, eventually to yield more ornithine and urea. Scientific recognition of his work followed almost immediately, and at the end of 1932—less than a year and a half after he began his research—Krebs found himself appointed as a *Privatdozent* at the University of Freiburg. He immediately embarked on the more ambitious project of identifying the intermediate steps in the metabolic breakdown of carbohydrates and fatty acids.

Krebs was not to enjoy his new position in Germany for long. In the spring of 1933, along with many other German scientists, he found himself dismissed from his job because of Nazi purging. Although Krebs had renounced the Jewish faith twelve years earlier at the urging of his patriotic father, who believed wholeheartedly in the assimilation of all German Jews, this legal declaration proved insufficiently strong for the Nazis. In June of 1933, he sailed for England to work in the **biochemistry** lab of Sir Frederick Gowland Hopkins of the Cambridge School of Biochemistry. Supported by a fellowship from the Rockefeller Foundation, Krebs resumed his research in the British laboratory. The following year, he augmented his research duties with the position of demonstrator in biochemistry. Laboratory space in Cambridge was cramped, however, and in 1935 Krebs was lured to the post of lecturer in the University of Sheffield's Department of Pharmacology by the prospect of more lab space, a semi-permanent appointment, and a salary almost double the one Cambridge was paying him.

His Sheffield laboratory established, Krebs returned to a problem that had long preoccupied him: how the body produced the essential amino acids that play such an important role in the metabolic process. By 1936, Krebs had begun to suspect that citric acid played an essential role in the oxidative metabolism by which the carbohydrate pyruvic acid is broken down so as to release energy. Together with his first Sheffield graduate student, William Arthur Johnson, Krebs observed a process akin to that in urea formation. The two researchers showed that even a small amount of citric acid could increase the oxygen absorption rate of living tissue. Because the amount of oxygen absorbed was greater than that needed to completely oxidize the citric acid, Krebs concluded that citric acid has a catalytic effect on the process of pyruvic acid conversion. He was also able to establish that the process is cyclical, citric acid being regenerated and replenished in a subsequent step. Although Krebs spent many more years refining the understanding of intermediary metabolism, these early results provided the key to the chemistry that sustains life processes. In June of 1937, he sent a letter to *Nature* reporting these preliminary findings. Within a week, the editor notified him that his paper could not be published without a delay. Undaunted, Krebs revised and expanded the paper and sent it to the new Dutch journal *Enzymologia,* which he knew would rapidly publicize this significant finding.

In 1938, Krebs married Margaret Fieldhouse, a teacher of domestic science in Sheffield. The couple eventually had three children. In the winter of 1939, the university named him lecturer in biochemistry and asked him to head their new department in the field. Married to an Englishwoman, Krebs became a naturalized English citizen in September, 1939, three days after World War II began.

The war affected Krebs's work minimally. He conducted experiments on vitamin deficiencies in conscientious objectors, while maintaining his own research on metabolic cycles. In 1944, the Medical Research Council asked him to head a new department of biological chemistry. Krebs refined his earlier discoveries throughout the war, particularly trying to determine how universal the Krebs cycle is among living organisms. He was ultimately able to establish that all organisms, even **microorganisms**, are sustained by the same chemical processes. These findings later prompted Krebs to speculate on the role of the metabolic cycle in **evolution**.

In 1953, Krebs received the Nobel Prize in physiology and medicine, which he shared with Fritz Lipmann, the discoverer of co-enzyme A. The following year, Oxford University offered him the Whitley professorship in biochemistry and the chair of its substantial department in that field. Once Krebs had ascertained that he could transfer his metabolic research unit to Oxford, he consented to the appointment. Throughout the next two decades, Krebs continued research into intermediary metabolism. He established how fatty acids are drawn into the metabolic cycle and studied the regulatory mechanism of intermediary metabolism. Research at the end of his life was focused on establishing that the metabolic cycle is the most efficient mechanism by which an organism can convert food to energy. When Krebs reached Oxford's mandatory retirement age of sixty-seven, he refused to end his research and made arrangements to move his research team to a laboratory established for him at the Radcliffe Hospital. Krebs died at the age of eighty-one.

See also Cell cycle and cell division; Cell membrane transport

KREBS CYCLE

The Krebs cycle is a set of biochemical reactions that occur in the mitochondria. The Krebs cycle is the final common pathway for the oxidation of food molecules such as sugars and fatty acids. It is also the source of intermediates in biosynthetic pathways, providing carbon skeletons for the synthesis of amino acids, nucleotides, and other key molecules in the cell. The Krebs cycle is also known as the citric acid cycle, and the tricarboxylic acid cycle. The Krebs cycle is a cycle because, during its course, it regenerates one of its key reactants.

To enter the Krebs cycle, a food molecule must first be broken into two- carbon fragments known as acetyl groups, which are then joined to the carrier molecule coenzyme A

(the A stands for acetylation). Coenzyme A is composed of the **RNA** nucleotide adenine diphosphate, linked to a pantothenate, linked to a mercaptoethylamine unit, with a terminal S-H.Dehydration of this linkage with the OH of an acetate group produces acetyl CoA. This reaction is catalyzed by pyruvate dehydrogenase complex, a large multienzyme complex.

The acetyl CoA linkage is weak, and it is easily and irreversibly hydrolyzed when Acetyl CoA reacts with the four-carbon compound oxaloacetate. Oxaloacetate plus the acetyl group form the six-carbon citric acid, or citrate. (Citric acid contains three carboxylic acid groups, hence the alternate names for the Krebs cycle.)

Following this initiating reaction, the citric acid undergoes a series of transformations. These result in the formation of three molecules of the high-energy hydrogen carrier NADH (nicotinamide adenine dinucleotide), 1 molecule of another hydrogen carrier FADH2 (flavin adenine dinucleotide), 1 molecule of high-energy GTP (guanine triphosphate), and 2 molecules of carbon dioxide, a waste product. The oxaloacetate is regenerated, and the cycle is ready to begin again. NADH and FADH2 are used in the final stages of cellular **respiration** to generate large amounts of ATP.

As a central metabolic pathway in the cell, the rate of the Krebs cycle must be tightly controlled to prevent too much, or too little, formation of products. This regulation occurs through inhibition or activation of several of the **enzymes** involved. Most notably, the activity of pyruvate dehydrogenase is inhibited by its products, acetyl CoA and NADH, as well as by GTP. This enzyme can also be inhibited by enzymatic addition of a phosphate group, which occurs more readily when ATP levels are high. Each of these actions serves to slow down the Krebs cycle when energy levels are high in the cell. It is important to note that the Krebs cycle is also halted when the cell is low on oxygen, even though no oxygen is consumed in it. Oxygen is needed further along in cell respiration though, to regenerate NAD+ and FAD. Without these, the cycle cannot continue, and pyruvic acid is converted in the cytosol to lactic acid by the **fermentation** pathway.

The Krebs cycle is also a source for precursors for biosynthesis of a number of cell molecules. For instance, the synthetic pathway for amino acids can begin with either oxaloacetate or alpha-ketoglutarate, while the production of porphyrins, used in hemoglobin and other proteins, begins with succinyl CoA. Molecules withdrawn from the cycle for biosynthesis must be replenished. Oxaloacetate, for instance, can be formed from pyruvate, carbon dioxide, and water, with the use of one ATP molecule.

See also Mitochondria and cellular energy

LABORATORY TECHNIQUES IN IMMUNOLOGY

Various laboratory techniques exist that rely on the use of antibodies to visualize components of **microorganisms** or other cell types and to distinguish one cell or organism type from another.

Electrophoresis is a technique whereby the protein or carbohydrate components of microorganisms can be separated based upon their migration through a gel support under the driving influence of electricity. Depending upon the composition of the gel, separation can be based on the net charge of the components or on their size. Once the components are separated, they can be distinguished immunologically. This application is termed **immunoelectrophoresis**.

Immunoelectrophoresis relies upon the exposure of the separated components in the gel to a solution that contains an **antibody** that has been produced to one of the separated proteins. Typically, the antibody is generated by the injection of the purified protein into an animal such as a rabbit. For example, the protein that comprises the flagellar appendage of a certain **bacteria** can be purified and injected into the rabbit, so as to produce rabbit anti-flagellar protein.

Immunoelectrophoresis can be used in a clinical **immunology** laboratory in order to diagnose illness, especially those that alter the immunoglobulin composition of body fluids. Research immunology laboratories also employ immunoelectrophoresis to analyze the components of organisms, including microorganisms.

One example of an immunoelectrophoretic technique used with microorganisms is known as the Western Blot. Proteins that have been separated on a certain type of gel support can be electrically transferred to a special membrane. Application of the antibody will produce binding between the antibody and the corresponding **antigen**. Then, an antibody generated to the primary antibody (for example, goat anti-rabbit antibody) is added. The secondary antibody will bind to the primary antibody. Finally, the secondary antibody can be constructed so that a probe binds to the antibody's free end. A chemical reaction produces a color change in the probe. Thus, bound primary antibody is visualized by the development of a dark band on the support membrane containing the electrophoretically separated proteins. Various controls can be invoked to ensure that this reaction is real and not the result of an experimental anomaly.

A similar reaction can be used to detect antigen in sections of biological material. This application is known as immunohistochemistry. The sections can be examined using either an **electron microscope** or a light **microscope**. The preparation techniques differ for the two applications, but both are similar in that they ensure that the antigen is free to bind the added antibody. Preservation of the antigen binding capacity is a delicate operation, and one that requires a skilled technician. The binding is visualized as a color reaction under light microscopic illumination or as an increased electron dense area under the electron beam of the electron microscope.

The binding between antigen and antibody can be enhanced in light microscopic immunohistochemistry by the exposure of the specimen to heat. Typically a microwave is used. The heat energy changes the configuration of the antigen slightly, to ease the fit of the antigen with the antibody. However, the shape change must not be too great or the antibody will not recognize the altered antigen molecule.

Another well-establish laboratory immunological technique is known as enzyme-linked immunosorbent assay. The technique is typically shortened to **ELISA**. In the ELISA technique, antigen is added to a solid support. Antibody is flooded over the support. Where an antibody recognizes a corresponding antigen, binding of the two will occur. Next an antibody raised against the primary antibody is applied, and binding of the secondary antibody to the primary molecule occurs. Finally, a substrate is bound to a free portion of the secondary antibody, and the binding can be subsequently visualized as a color reaction. Typically, the ELISA test is

Titration burettes are used to carefully control the pH of solutions used in laboratory procedures.

done using a plastic plate containing many small wells. This allows up to 100 samples to be tested in a single experiment. ELISA can reveal the presence of antigen in fluids such as a patient's serum, for example.

The nature of the antibody can be important in laboratory immunological techniques. Antibodies such as those raised in a rabbit or a goat are described as being polyclonal in nature. That is, they do recognize a certain antigenic region. But if that region is present on different molecules, the antibody will react with all the molecules. The process of monoclonal antibody production can make antigenic identification much more specific, and has revolutionized immunological analysis.

Monoclonal antibodies are targeted against a single antigenic site. Furthermore, large amounts of the antibody can be made. This is achieved by fusing the antibody-producing cell obtained from an immunized mouse with a tumor cell. The resulting hybrid is known as a hybridoma. A particular hybridoma will mass-produce the antibody and will express the antibody on the surface of the cell. Because hybridoma cells are immortal, they grow and divide indefinitely. Hence the production of antibody can be ceaseless.

Monoclonal antibodies are very useful in a clinical immunology laboratory, as an aid to diagnose diseases and to detect the presence of foreign or abnormal components in the blood. In the research immunology laboratory, monoclonal technology enables the specific detection of an antigenic target and makes possible the development of highly specific vaccines.

One example of the utility of monoclonal antibodies in an immunology laboratory is their use in the technique of flow cytometry. This technique separates sample as individual sample molecules pass by a detector. Sample can be treated with monoclonal antibody followed by a second treatment with an antibody to the monoclonal to which is attached a molecule that will fluoresce when exposed to a certain wavelength of light. When the labeled sample passes by the detector and is illuminated (typically by laser light of the pre-determined wavelength), the labeled sample molecules will fluoresce. These can be detected and will be shunted off to a special collection receptacle. Many sorts of analyses are possible using flow cytometry, from the distinguishing of one type of bacteria from another to the level of the genetic material comprising such samples.

See also Antibody-antigen, biochemical and molecular reactions

LABORATORY TECHNIQUES IN MICROBIOLOGY

A number of techniques are routine in microbiology laboratories that enable **microorganisms** to be cultured, examined and identified.

An indispensable tool in any microbiology laboratory is the inoculating loop. The loop is a piece of wire that is looped at one end. By heating up the loop in an open flame, the loop can be sterilized before and after working with **bacteria**. Thus, **contamination** of the bacterial sample is minimized. The inoculating loop is part of what is known as aseptic (or sterile) technique.

Another staple piece of equipment is called a petri plate. A petri plate is a sterile plastic dish with a lid that is used as a receptacle for solid growth media.

In order to diagnose an infection or to conduct research using a microorganism, it is necessary to obtain the organism in a pure **culture**. The streak plate technique is useful in this regard. A sample of the bacterial population is added to one small region of the growth medium in a petri plate and spread in a back and forth motion across a sector of the plate using a sterile inoculating loop. The loop is sterilized again and used to drag a small portion of the culture across another sector of the plate. This acts to dilute the culture. Several more repeats yield individual colonies. A **colony** can be sampled and streaked onto another plate to ensure that a pure culture is obtained.

Dilutions of bacteria can be added to a petri plate and warm growth medium added to the aliquot of culture. When the medium hardens, the bacteria grow inside of the **agar**. This is known as the pour plate technique, and is often used to determine the number of bacteria in a sample. Dilution of the original culture of bacteria is often necessary to reach a countable level.

Bacterial numbers can also be determined by the number of tubes of media that support growth in a series of dilutions of the culture. The pattern of growth is used to determine what is termed the most probable number of bacteria in the original sample.

As a bacterial population increases, the medium becomes cloudier and less light is able to pass through the culture. The optical density of the culture increases. A relationship between the optical density and the number of living bacteria determined by the viable count can be established.

The growth sources for microorganisms such as bacteria can be in a liquid form or the solid agar form. The composition of a particular medium depends on the task at hand. Bacteria are often capable of growth on a wide variety of media, except for those bacteria whose nutrient or environmental requirements are extremely restricted. So-called non-selective media are useful to obtain a culture. For example, in **water quality** monitoring, a non-selective medium is used to obtain a total enumeration of the sample (called a heterotrophic plate count). When it is desirable to obtain a specific bacterial species, a selective medium can be used. Selective media support the growth of one or a few bacterial types while excluding the growth of other bacteria. For example, the growth of the bacterial genera *Salmonella* and *Shigella* are selectively encouraged by the use of *Salmonella-Shigella* agar. Many selective media exist.

Lab technician performing medical research.

Liquid cultures of bacteria can be nonspecific or can use defined media. A batch culture is essentially a stopped flask that is about one third full of the culture. The culture is shaken to encourage the diffusion of oxygen from the overlying air into the liquid. Growth occurs until the nutrients are exhausted. Liquid cultures can be kept growing indefinitely by adding fresh medium and removed spent culture at controlled rates (a chemostat) or at rates that keep the optical density of the culture constant (a turbidostat). In a chemostat, the rate at which the bacteria grow depends on the rate at which the critical nutrient is added.

Living bacteria can also be detected by direct observation using a light **microscope**, especially if the bacteria are capable of the directed movement that is termed motility. Also, living microorganisms are capable of being stained in certain distinctive ways by what are termed vital stains. Stains can also be used to highlight certain structures of bacteria, and even to distinguish certain bacteria from others. One example is the Gram's stain, which classifies bacteria into two camps, Gram positive and Gram negative. Another example is the Ziehl-Neelsen stain, which preferentially stains the cell wall of a type of bacteria called Mycobacteria.

Techniques also help detect the presence of bacteria that have become altered in their structure or genetic composition. The technique of replica plating relies on the adhesion of microbes to the support and the transfer of the microbes to a series of growth media. The technique is analogous to the making of photocopies of an original document. The various media can be tailored to detect a bacteria that can grow in the presence of a factor, such as an antibiotic, that the bacteria from the original growth culture cannot tolerate.

Various biochemical tests are utilized in a microbiology laboratory. The ability of a microbe to utilize a particular compound and the nature of the compound that is produced are important in the classification of microorganisms, and the diagnosis of infections. For example, coliform bacteria were traditionally identified by a series of biochemical reactions that formed a presumptive-confirmed-completed triad of tests. Now, media have been devised that specifically support the growth of coliform bacteria, and ***Escherichia coli*** in particular.

Various laboratory tests are conducted in animals to obtain an idea of the behavior of microorganisms *in vivo*. One such test is the lethal dose 50 (LD50), which measures the amount of an organism or its toxic components that will kill 50 percent of the test population. The lower the material necessary to achieve the LD50, the more potent is the disease component of organism.

See also Antibiotic resistance, tests for; Blood agar, hemolysis, and hemolytic reactions; Microscopy; Qualitative and quantitative analysis in microbiology

LACTIC ACID BACTERIA

Lactic acid **bacteria** compose a group of bacteria that degrade carbohydrate (e.g., **fermentation**) with the production of lactic acid. Examples of genera that contain lactic acid bacteria include *Streptococcus*, *Lactobacillus*, *Lactococcus*, and *Leuconostoc*.

The production of lactic acid has been used for a long time in food production (e.g., yogurt, cheese, sauerkraut, sausage,). Since the 1970s, the popularity of fermented foods such as kefir, kuniss, and tofu that were formally confined to certain ethnically oriented cuisines, has greatly increased.

Generally, lactic acid bacteria are Gram-positive bacteria that do not form spores and which are able to grow both in the presence and absence of oxygen. Another common trait of lactic acid bacteria is their inability to manufacture the many compounds that they need to survive and grow. Most of the nutrients must be present in the environment in which the bacteria reside. Their fastidious nutritional needs restrict the environments in which lactic acid bacteria exist. The mouth and intestinal tract of animals are two such environments, where the lactic acid bacterium *Enterococcus faecalis* lives. Other environments include plant leaves (*Leuconostoc*, *Lactobacillus*, and decaying organic material.

The drop in **pH** that occurs as lactic acid is produced by the bacteria is beneficial in the preservation of food. The lowered pH inhibits the growth of most other food spoilage **microorganisms**. Abundant growth of the lactic acid bacteria, and so production of lactic acid, is likewise hindered by the low pH. The low pH environment prolongs the shelf life of foods (e.g., pickles, yogurt, cheese) from **contamination** by bacteria that are common in the kitchen (e.g., *Escherichia coli,* or bacteria that are able to grow at refrigeration temperatures (e.g., *Listeria*. The drop in the oxygen level during lactic acid fermentation is also an inhibitory factor for potential food pathogens. Research is actively underway to extend the protection afforded by lactic acid bacteria to others foods, such as vegetables.

The acidity associated with lactic acid bacteria has also been useful in preventing colonization of surfaces with infectious bacteria. The best example of this is the vagina. Colonization of the vaginal epithelial cells with *Lactobacillus* successfully thwarts the subsequent colonization of the cell surface with harmful bacteria, thus reducing the incidence of chronic vaginal **yeast** infections.

Lactic acid bacteria produce antibacterial compounds that are known as bacteriocins. Bacteriocins act by punching holes through the membrane that surrounds the bacteria. Thus, bacteriocins activity is usually lethal to the bacteria. Examples of bacteriocins are nisin and leucocin. Nisin inhibits the growth of most gram-positive bacteria, particularly spore-formers (e.g., *Clostridium botulinum*. This bacteriocin has been approved for use as a food preservative in the United States since 1989. Leucocin is inhibitory to the growth of *Listeria monocytogenes*.

Lactic acid bacteria are also of economic importance in the preservation of agricultural crops. A popular method of crop preservation utilizes what is termed silage. Silage is essentially the exposure of crops (e.g., grasses, corn, alfalfa) to lactic acid bacteria. The resulting fermentation activity lowers the pH on the surface of the crop, preventing colonization of the crop by unwanted microorganisms.

See also Economic uses and benefits of microorganisms

LACTOBACILLUS

Lactobacillus is the name given to a group of Gram-negative **bacteria** that do not form spores but derive energy from the conversion of the sugar glucose into another sugar known as lactose. The name of the genus derives from the distinctive sugar use. *Lactobacillus* has a number of commercial uses, especially in aspects of dairy production, including the manufacture of yogurt. As well, *Lactobacillus* is part of the normal microbial population of the human adult vagina, where it exerts a protective effect.

Prominent examples of the genus include *Lactobacillus acidophilus*, *Lactobacillus* GG, *Bifidobacterium bifidum*, and *Bifidobacterium longum*.

A distinctive feature of the members of the genus *Lactobacillus* is the formation of lactic acid from glucose. This is the property that confers the sour taste to natural, *Lactobacillus*-containing yogurt. As well, the lactic acid lowers the **pH** of the environment that the bacteria dwell in. In the case of the vagina, this acidic change can inhibit the growth of other, harmful invading bacteria. Consistent with this, the use of suppositories containing *Lactobacillus* species has been successful in controlling recurrent bacterial vaginal infections. Similarly, use of the bacterium has been promising in the control and prevention of recurrent urinary tract infections.

Aside from the exclusion of bacteria due to the pH alteration in the vagina or urinary tract, *Lactobacillus* also adheres to cells lining the vagina and the urinary tract, and colonizes

these surfaces. The luxuriant growth of these bacteria excludes other bacteria from gaining a foothold. This phenomenon is known as competitive exclusion.

Commercially, *Lactobacillus* is best known as the basis of yogurt manufacture. A mixture of *Lactobacillus bulgaricus* or *Lactobacillus acidophilus* and *Streptococcus thermophilus* produce the lactic acid that ferments milk.

Yogurt that contains live bacteria usually contains *Lactobacillus acidophilus*. There is evidence that the persistence of the bacteria in the intestinal tract for up to a week after consuming yogurt increases the number of antibody-secreting cells in the intestine. Also *Lactobacillus acidophilus* bacteria possess and enzyme called lactase that enables the bacteria are to utilize undigested starches, particularly those in milk, that would otherwise be eliminated from the body.

Yet another benefit of *Lactobacillus* is the production of beneficial compounds that are used by the body. For example, *Lactobacillus acidophilus* produces niacin, folic acid, and pyridoxine, a group of compounds that collectively are referred to as the B vitamins.

Another noteworthy strain of *Lactobacillus* is known as *Lactobacillus GG*. This strain was isolated from humans in the 1980s by Drs. Sherwood Gorbach and Barry Goldin. The initials of their last names are the basis for the GG designation. *Lactobacillus GG* has shown great promise as a nutritional supplement because the bacteria are able to survive the passage through the very acidic conditions of the stomach. They then colonize the intestinal tract. There, the bacteria produce a compound that has antibacterial activity. This may help maintain the intestinal tract free from invading bacteria.

See also Microbial flora of the stomach and gastrointestinal tract; Probiotics

LANCEFIELD, REBECCA CRAIGHILL (1895-1981)

American bacteriologist

Rebecca Craighill Lancefield is best-known throughout the scientific world for the system she developed to classify the **bacteria** *Streptococcus*. Her colleagues called her laboratory at the Rockefeller Institute for Medical Research (now Rockefeller University) "the Scotland Yard of streptococcal mysteries." During a research career that spanned six decades, Lancefield meticulously identified over fifty types of this bacteria. She used her knowledge of this large, diverse bacterial family to learn about pathogenesis and **immunity** of its afflictions, ranging from sore throats, rheumatic fever and scarlet fever, to heart and kidney disease. The Lancefield system remains a key to the medical understanding of streptococcal diseases.

Born Rebecca Craighill on January 5, 1895, in Fort Wadsworth on Staten Island in New York, she was the third of six daughters. Her mother, Mary Montague Byram, married William Edward Craighill, a career army officer in the Army Corps of Engineers who had graduated from West Point. Lancefield received a bachelor's degree in 1916 from Wellesley College, after changing her major from English to zoology. Two years later, she earned a master's degree from Columbia University, where she pursued bacteriology in the laboratory of Hans Zinsser. Immediately upon graduating from Columbia, she formed two lifelong partnerships. She married Donald Lancefield, who had been a classmate of hers in a genetics class. She was also hired by the Rockefeller Institute to help bacteriologists Oswald Avery and Alphonse Dochez, whose expertise on *Pneumococcus* was then being applied to a different bacterium. This was during World War I, and the project at Rockefeller was to discover whether distinct types of *Streptococci* could be isolated from soldiers in a Texas epidemic so that a serum might be produced to prevent infection. The scientists employed the same serological techniques that Avery had used to distinguish types of *Pneumococcus*. Within a year, Avery, Dochez, and Lancefield had published a major report which described four types of *Streptococcus*. This was Lancefield's first paper.

Lancefield and her husband took a short hiatus to teach in his home state at the University of Oregon, then returned to New York. Lancefield worked simultaneously on a Ph.D. at Columbia and on rheumatic fever studies at the Rockefeller Institute in the laboratory of Homer Swift, and her husband joined the Columbia University faculty in biology. Before World War I, physicians had suspected that *Streptococcus* caused rheumatic fever. But scientists, including Swift, had not been able to recover a specific organism from patients. Nor could they reproduce the disease in animals using patient cultures. Lancefield's first project with Swift, which was also her doctoral work, showed that the alpha-hemolytic class of *Streptococcus,* also called green or viridans, was not the cause of rheumatic fever.

As a result of her work with Swift, Lancefield decided that a more basic approach to rheumatic fever was needed. She began sorting out types among the disease-causing class, the beta-hemolytic **streptococci**. She used serological techniques while continuing to benefit from Avery's advice. Her major tool for classifying the bacteria was the precipitin test. This involved mixing soluble type-specific antigens, or substances used to stimulate immune responses, with antisera (types of serum containing antibodies) to give visible precipitates. Precipitates are the separations of a substance, in this case bacteria, from liquid in a solution, the serum, in order to make it possible to study the bacteria on its own.

Lancefield soon recovered two surface antigens from these streptococci. One was a polysaccharide, or carbohydrate, called the C substance. This complex sugar molecule is a major component of the cell wall in all streptococci. She could further subdivide its dissimilar compositions into groups and she designated the groups by the letters A through O. The most common species causing human disease, *Streptococcus pyogenes,* were placed in group A. Among the group A streptococci, Lancefield found another **antigen** and determined it was a protein, called M for its matt appearance in **colony** formations. Because of differences in M protein composition, Lancefield was able to subdivide group A streptococci into types. During

her career, she identified over fifty types, and since her death in 1981, bacteriologists have identified thirty more.

Lancefield's classification converged with another typing system devised by Frederick Griffith in England. His typing was based on a slide agglutination method, in which the bacteria in the serum collect into clumps when an **antibody** is introduced. For five years the two scientists exchanged samples and information across the Atlantic Ocean, verifying each other's types, until Griffith's tragic death during the bombing of London in 1940. Ultimately, Lancefield's system, based on the M types, was chosen as the standard for classifying group A streptococci.

In further studies on the M protein, Lancefield revealed this antigen is responsible for the bacteria's virulence because it inhibits **phagocytosis**, thus keeping the white blood cells from engulfing the streptococci. This finding came as a surprise, because Avery had discovered that virulence in the *Pneumococcus* was due to a polysaccharide, not a protein. Lancefield went on to show the M antigen is also the one that elicits protective immune reactions.

Lancefield continued to group and type strep organisms sent from laboratories around the world. Until the end of her life her painstaking investigations helped unravel the complexity and diversity of these bacteria. Her thoroughness was a significant factor in her small but substantial bibliography of nearly sixty papers.

Once her system of classification was in place, however, Lancefield returned to her original quest to elucidate connections between the bacteria's constituents and the baffling nature of streptococcal diseases. She found that a single serotype of group A can cause a variety of streptococcal diseases. This evidence reversed a long-standing assumption that every disease must be caused by a specific microbe. Also, because the M protein is type-specific, she found that acquired immunity to one group A serotype could not protect against infections caused by others in group A.

From her laboratory at Rockefeller Hospital, Lancefield could follow patient records for very long periods. She conducted a study that determined that once immunity is acquired to a serotype, it can last up to thirty years. This particular study revealed the unusual finding that high titers, or concentrations, of antibody persist in the absence of antigen. In the case of rheumatic fever, Lancefield illustrated how someone can suffer recurrent attacks, because each one is caused by a different serotype.

In other studies, Lancefield focused on antigens. She and Gertrude Perlmann purified the M protein in the 1950s. Twenty years later she developed a more conservative test for typing it and continued characterizing other group A protein antigens designated T and R. Ten years after her official retirement, she made a vital contribution on the group B streptococci. She clarified the role of their polysaccharides in virulence and showed how protein antigens on their surface also played a protective role. During the 1970s, an increasingly high-rate of infants were born with group B **meningitis**, and her work laid the basis for the medical response to this problem.

During World War II, Lancefield had performed special duties on the Streptococcal Diseases Commission of the Armed Forces Epidemiological Board. Her task involved identifying strains and providing antisera for **epidemics** of scarlet and rheumatic fever among soldiers in military camps. After the commission dissolved, her colleagues in the "Strep Club" created the Lancefield Society in 1977, which continues to hold regular international meetings on advances in streptococcal research.

An associate member at Rockefeller when **Maclyn McCarty** took over Swift's laboratory in 1946, Lancefield became a full member and professor in 1958, and emeritus professor in 1965. While her career and achievements took place in a field dominated by men, Lewis Wannamaker in American Society for Microbiology News quotes Lancefield as being "annoyed by any special feeling about women in science." Nevertheless, most recognition for Lancefield came near her retirement. In 1961, she was the first woman elected president of the American Association of Immunologists, and in 1970, she was one of few women elected to the National Academy of Sciences. Other honors included the T. Duckett Jones Memorial Award in 1960, the American Heart Association Achievement Award in 1964, the New York Academy of Medicine Medal in 1973, and honorary degrees from Rockefeller University in 1973 and Wellesley College in 1976.

In addition to her career as a scientist, Lancefield had one daughter. Lancefield was devoted to research and preferred not to go on lecture tours or attend scientific meetings. Rockefeller's laboratories were not air-conditioned and her main diversion was leaving them during the summer and spending the entire season in Woods Hole, Massachusetts. There she enjoyed tennis and swimming with her family, which eventually included two grandsons. Official retirement did not change her lifestyle. She drove to her Rockefeller laboratory from her home in Douglaston, Long Island, every working day until she broke her hip in November 1980. She died of complications from this injury on March 3, 1981, at the age of eighty-six.

The pathogenesis of rheumatic fever still eludes scientists, and **antibiotics** have not eliminated streptococcal diseases. Yet the legacy of Lancefield's system and its fundamental links to disease remain and a **vaccine** against several group A streptococci is being developed in her former laboratory at Rockefeller University by Vincent A. Fischetti.

See also Bacteria and bacterial infection; Streptococci and streptococcal infections

LANDSTEINER, KARL (1868-1943)

American immunologist

Karl Landsteiner was one of the first scientists to study the physical processes of **immunity**. He is best known for his identification and characterization of the human blood groups, A, B, and O, but his contributions spanned many areas of **immunology**, bacteriology, and pathology over a prolific forty-year career. Landsteiner identified the agents responsible for immune reactions, examined the interaction of antigens and antibodies, and studied allergic reactions in experimental ani-

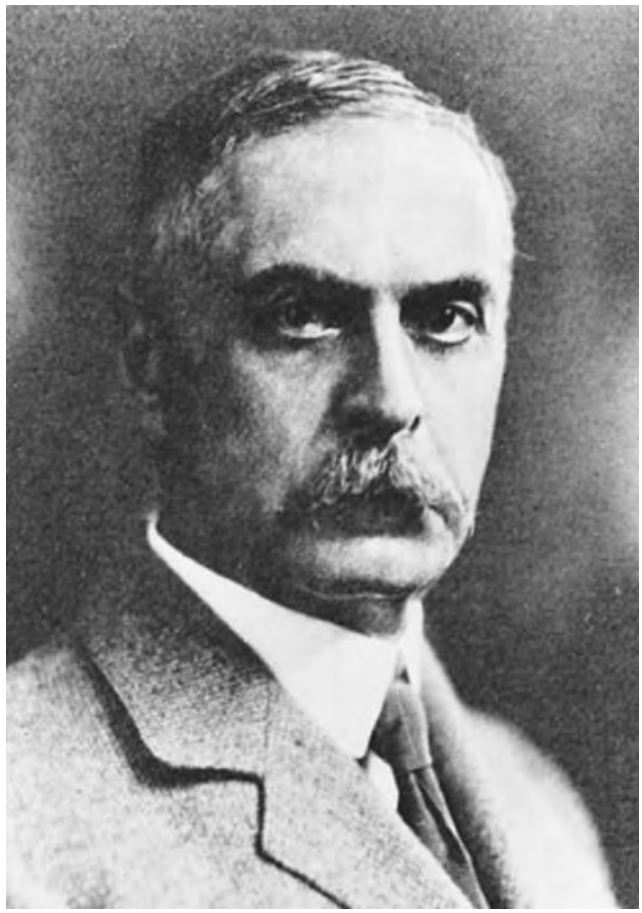
mals. He determined the viral cause of **poliomyelitis** with research that laid the foundation for the eventual development of a polio **vaccine**. He also discovered that some simple chemicals, when linked to proteins, produced an immune response. Near the end of his career in 1940, Landsteiner and immunologist Philip Levine discovered the **Rh** factor that helped save the lives of many unborn babies whose Rh factor did not match their mothers. For his work identifying the human blood groups, Landsteiner was awarded the Nobel Prize for medicine in 1930.

Karl Landsteiner was born on in Vienna, Austria. In 1885, at the age of 17, Landsteiner passed the entrance examination for medical school at the University of Vienna. He graduated from medical school at the age of 23 and immediately began advanced studies in the field of organic chemistry, working in the research laboratory of his mentor, Ernst Ludwig. In Ludwig's laboratory Landsteiner's interest in chemistry blossomed into a passion for approaching medical problems through a chemist's eye.

For the next ten years, Landsteiner worked in a number of laboratories in Europe, studying under some of the most celebrated chemists of the day: Emil Fischer, a protein chemist who subsequently won the Nobel Prize for chemistry in 1902, in Wurzburg; Eugen von Bamberger in Munich; and Arthur Hantzsch and Roland Scholl in Zurich. Landsteiner published many journal articles with these famous scientists. The knowledge he gained about organic chemistry during these formative years guided him throughout his career. The nature of antibodies began to interest him while he was serving as an assistant to **Max von Gruber** in the Department of **Hygiene** at the University of Vienna from 1896 to 1897. During this time Landsteiner published his first article on the subject of bacteriology and **serology**, the study of blood.

Landsteiner moved to Vienna's Institute of Pathology in 1897, where he was hired to perform autopsies. He continued to study immunology and the mysteries of blood on his own time. In 1900, Landsteiner wrote a paper in which he described the agglutination of blood that occurs when one person's blood is brought into contact with that of another. He suggested that the phenomenon was not due to pathology, as was the prevalent thought at the time, but was due to the unique nature of the individual's blood. In 1901, Landsteiner demonstrated that the blood serum of some people could clump the blood of others. From his observations he devised the idea of mutually incompatible blood groups. He placed blood types into three groups: A, B, and C (later referred to as O). Two of his colleagues subsequently added a fourth group, AB.

In 1907, the first successful transfusions were achieved by Dr. Reuben Ottenberg of Mt. Sinai Hospital, New York, guided by Landsteiner's work. Landsteiner's accomplishment saved many lives on the battlefields of World War I, where transfusion of compatible blood was first performed on a large scale. In 1902, Landsteiner was appointed as a full member of the Imperial Society of Physicians in Vienna. That same year he presented a lecture, together with Max Richter of the Vienna University Institute of Forensic Medicine, in which the two reported a new method of typing dried blood stains to help solve crimes in which blood stains are left at the scene.



Karl Landsteiner, awarded the 1930 Nobel Prize in Medicine or Physiology for his discovery of human blood groups.

In 1908, Landsteiner took charge of the department of pathology at the Wilhelmina Hospital in Vienna. His tenure at the hospital lasted twelve years, until March of 1920. During this time, Landsteiner was at the height of his career and produced 52 papers on serological immunity, 33 on bacteriology and six on pathological anatomy. He was among the first to dissociate antigens that stimulate the production of immune responses known as antibodies, from the antibodies themselves. Landsteiner was also among the first to purify antibodies, and his purification techniques are still used today for some applications in immunology.

Landsteiner also collaborated with Ernest Finger, the head of Vienna's Clinic for Venereal Diseases and Dermatology. In 1905, Landsteiner and Finger successfully transferred the venereal disease **syphilis** from humans to apes. The result was that researchers had an animal model in which to study the disease. In 1906, Landsteiner and Viktor Mucha, a scientist from the Chemical Institute at Finger's clinic, developed the technique of dark-field microscopy to identify and study the **microorganisms** that cause syphilis.

One day in 1908, the body of a young polio victim was brought in for autopsy. Landsteiner took a portion of the boy's spinal column and injected it into the spinal canal of several

species of experimental animals, including rabbits, guinea pigs, mice, and monkeys. Only the monkeys contracted the disease. Landsteiner reported the results of the experiment, conducted with Erwin Popper, an assistant at the Wilhelmina Hospital.

Scientists had accepted that polio was caused by a microorganism, but previous experiments by other researchers had failed to isolate a causative agent, which was presumed to be a bacterium. Because monkeys were hard to come by in Vienna, Landsteiner went to Paris to collaborate with a Romanian bacteriologist, Constantin Levaditi of the Pasteur Institute. Working together, the two were able to trace poliomyelitis to a virus, describe the manner of its transmission, time its incubation phase, and show how it could be neutralized in the laboratory when mixed with the serum of a convalescing patient. In 1912, Landsteiner proposed that the development of a vaccine against poliomyelitis might prove difficult but was certainly possible. The first successful polio vaccine, developed by **Jonas Salk**, wasn't administered until 1955.

Landsteiner accepted a position as chief dissector in a small Catholic hospital in The Hague, Netherlands where he performed routine laboratory tests on urine and blood from 1919 to 1922. During this time he began working on the concept of haptens, small molecular weight chemicals such as fats or sugars that determine the specificity of antigen-antibody reactions when combined with a protein carrier. He combined haptens of known structure with well-characterized proteins such as albumin, and showed that small changes in the hapten could affect **antibody** production. He developed methods to show that it is possible to sensitize animals to chemicals that cause contact dermatitis (**inflammation** of the skin) in humans, demonstrating that contact dermatitis is caused by an antigen-antibody reaction. This work launched Landsteiner into a study of the phenomenon of allergic reactions.

In 1922, Landsteiner accepted a position at the Rockefeller Institute in New York. Throughout the 1920s Landsteiner worked on the problems of immunity and allergy. He discovered new blood groups: M, N, and P, refining the work he had begun 20 years before. Soon after Landsteiner and his collaborator, Philip Levine, published the work in 1927, the types began to be used in paternity suits.

In 1929, Landsteiner became a United States citizen. He won the Nobel Prize for medicine in 1930 for identifying the human blood types. In his Nobel lecture, Landsteiner gave an account of his work on individual differences in human blood, describing the differences in blood between different species and among individuals of the same species. This theory is accepted as fact today but was at odds with prevailing thought when Landsteiner began his work. In 1936, Landsteiner summed up his life's work in what was to become a medical classic: *Die Spezifität der serologischen Reaktionen*, which was later revised and published in English, under the title *The Specificity of Serological Reactions*.

Landsteiner retired in 1939, at the age of seventy-one, but continued working in immunology. With Levine and Alexander Wiener he discovered another blood factor, labeled the Rh factor, for Rhesus monkeys, in which the factor was first discovered. The Rh factor was shown to be responsible for the infant disease, erythroblastosis fetalis that occurs when mother and fetus have incompatible blood types and the fetus is injured by the mother's antibodies. Landsteiner died in 1943, at the age of 75.

See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Blood agar, hemolysis, and hemolytic reactions; History of immunology; Rh and Rh incompatibility

LATENT VIRUSES AND DISEASES

Latent **viruses** are those viruses that can incorporate their genetic material into the genetic material of the infected host cell. Because the viral genetic material can then be replicated along with the host material, the virus becomes effectively "silent" with respect to detection by the host. Latent viruses usually contain the information necessary to reverse the latent state. The viral genetic material can leave the host genome to begin the manufacture of new virus particles.

The molecular process by which a virus becomes latent has been explored most fully in the **bacteriophage** designated lambda. The lysogenic process is complex and involves the interplay between several proteins that influence the **transcription** of genes that either maintain the latent state or begin the so-called lytic process, where the manufacture of new virus begins.

Bacteriophage lambda is not associated with disease. However, other viruses that can establish a latent relationship with the host are capable of causing disease. Examples of viruses include the **Herpes** Simplex Virus 1 (also dubbed HSV 1) and **retroviruses**. The latter group of viruses includes the **Human Immunodeficiency Viruses** (**HIVs**) that are the most likely cause of acquired immunodeficiency syndrome (**AIDS**).

In the case of HSV 1, the virus can become latent early in life, when many people are infected with the virus. The virus infects the mucous membranes located around the mouth. From this location the virus spreads to a region of certain nerve cells called the ganglion. It is here that the viral genetic material (**deoxyribonucleic acid**, or **DNA**) integrates into the host genetic material. The period of latency can span decades. Then, if the host is stressed such that the survival of the infected cells is in peril, the viral DNA is activated. The new virus particles migrate back to the mucous membranes of the mouth, where they erupt as "cold sores". A form of the reactivated herpes virus that is known as Herpes Zoster causes the malady of shingles. The painful sores associated with shingles can appear all over the body.

The re-emergence of HSV 1 later in life does qualify as a disease. However, it has been argued that the near universal prevalence of the latent form of the viral DNA in people worldwide qualifies HSV as being part of the normal microbial makeup of humans. Others argue that even the latent HSV state qualifies as an infection, albeit an infection that displays no symptoms and is essentially harmless to the host.

Other examples of a latent virus include the HIVs. The latent form of **HIV** is particularly insidious from the point of

view of treatment, because the drugs traditionally given to treat AIDS are effective only against the actively replicating form of the virus. In the absence of detectable virus, drug therapy may be discontinued. Then, if the virus is stimulated to leave the latent state and begin another round of infection, that infection will be uncontrolled. Indeed, it has been shown that even the near continuous administration of anti-HIV drugs does not completely eliminate the pool of latent virus in the **immune system**.

A hallmark of latent viral infections is that the immune system is not stimulated to respond. Indeed, with little or no viral products or new virus produced, the immune system has no target. This complicates the development of vaccines to infections such as HSV 1 and AIDS, because the nature of the **vaccine** effect is the stimulation of the immune system.

One experimental approach that is being explored with latent viral infections is to establish whether there is some aspect of the host cell that predisposes the cells to infection with a virus capable of becoming latent. Identification of such host factors could help in the design of therapeutic strategies to target these cells against viral infection.

See also Lysogeny; Virology; Viral genetics

LEDERBERG, JOSHUA (1925-)

American geneticist

Joshua Lederberg is a Nobel Prize-winning American geneticist whose pioneering work on genetic **recombination** in **bacteria** helped propel the field of **molecular genetics** into the forefront of biological and medical research. In 1946, Lederberg, working with **Edward Lawrie Tatum**, showed that bacteria may reproduce sexually, disproving the widely held theory that bacteria were asexual. The two scientists' discovery also substantiated that bacteria possess genetic systems comparable to those of higher organisms, thus providing a new repertoire for scientists to study the genetic basis of life.

Continuing with his work in bacteria, Lederberg also discovered the phenomena of genetic **conjugation** and **transduction**, or the transfer of either the entire **complement** of **chromosomes** or chromosome fragments, respectively, from cell to cell. In his work on conjugation and transduction, Lederberg became the first scientist to manipulate genetic material, which had far-reaching implications for subsequent efforts in genetic engineering and **gene** therapy. In addition to his laboratory research, Lederberg lectured widely on the complex relationship between science and society and served as a scientific adviser on **biological warfare** to the **World Health Organization**.

Lederberg was born in Montclair, New Jersey. His family moved to New York City where he attended Stuyvesant High School. Through a program known as the American Institute Science Laboratory, Lederberg was given the opportunity to conduct original research in a laboratory after school hours and on weekends. Here he pursued his interest in biology, working in cytochemistry, or the chemistry of cells. Lederberg was influenced early on by science-oriented writers such as Bernard Jaffe, Paul de Kruif, and H. G. Wells.

After graduating from high school in 1941, Lederberg entered Columbia University as a premedical student. He received a tuition scholarship from the Hayden Trust, which, coupled with living at home and commuting to school, made it financially possible for him to attend college. Although his undergraduate studies focused on zoology, Lederberg also received a foundation in humanistic studies under Lionel Trilling, James Gutman, and others. H. Burr Steinbach fostered Lederberg's work in zoology and helped him obtain a space in a histology lab where he could pursue his own research. This early undergraduate research included the cytophysiology of mitosis in plants and the uses of genetic analysis in cell biology. In 1942, Lederberg met Francis Ryan, whose work in the biochemical genetics of ***Neurospora*** (a genus of **fungi**) was Lederberg's first opportunity to see significant scientific research as it occurred. Lederberg graduated with honors in 1944 with a B.A. at the age of nineteen.

At the age of seventeen, Lederberg enlisted in the United States Navy V–12 college training program, which featured a condensed pre-med and medical curriculum to produce medical officers for the armed services during World War II. During his years as an undergraduate, he was also assigned duty to the U.S. Naval Hospital at St. Albans in Long Island. He began his medical courses at Columbia College of Physicians and Surgeons in 1944, but left after two years to study under Edward L. Tatum in the microbiology department at Yale University.

Tatum, with George W. Beadle, had made substantial contributions to biochemical genetics, including investigations proving that the **DNA** (**deoxyribonucleic acid**) of *Neurospora* played a fundamental role in many of the chemical reactions in *Neurospora* cells. Lederberg was interested in natural **selection** and helped Tatum continue his studies of *Neurospora*. Eventually, Lederberg and Tatum proceeded to embark on a more tenuous line of research, studying ***Escherichia coli*** (a bacterium that lives in the gastrointestinal tract) for evidence of genetic inheritance. At the international Cold Spring Harbor Symposium of 1946, Lederberg and Tatum presented their research on *E. coli* in addition to the *Neurospora* studies. An audience that included the leading molecular biologists and geneticists in the world met the scientists' announcement that they had discovered sexual or genetic recombination in the bacterium with keen interest. The prevailing theory among biologists of the time was that bacteria reproduced asexually by cells essentially splitting, creating two cells with a complete set of chromosomes (threadlike structures in the cell **nucleus** that carry genetic information). Lederberg and Tatum had found evidence that some strains of *E. coli* pass on hereditary material cell to cell. They found that a conjugation of two cells produced a cell that subsequently began dividing into offspring cells. These offspring showed that they inherited traits from each of the parent strains. Lederberg received requests for *E. coli* cultures by others who wanted to investigate his findings.

Joshua Lederberg (left) who, with his wife Esther (right), discovered the process of bacterial recombination. He was awarded the 1958 Nobel Prize in Medicine for this and other discoveries.

In 1947, while at Yale, Lederberg received an offer from the University of Wisconsin to become an assistant professor of genetics. Although only two years away from receiving his M.D. degree, Lederberg accepted the position at Wisconsin and received his Ph.D. degree from Yale in 1948. He worked at the University of Wisconsin for a decade after abandoning his medical training, although he noted his later honorary medical degrees from Tufts University and the University of Turin as being among his most valued.

Lederberg continued to make groundbreaking discoveries at Wisconsin that firmly established him as one of the most promising young intellects in the burgeoning field of genetics. By perfecting a method to isolate mutant bacteria species using ultraviolet light, Lederberg was able to prove the long-held theory that genetic **mutations** occurred spontaneously. He found he could mate two strains of bacteria, one resistant to **penicillin** and the other to streptomycin, and produce bacteria resistant to both **antibiotics**. He also found that he could manipulate a virus's virulence.

Working with graduate student Norton Zinder, Lederberg discovered genetic transduction, which involves the transfer only of hereditary fragments of information between cells as opposed to complete chromosomal replication (conjugation). Lederberg went on to breed unique strains of **viruses**. Although these strains promised to reveal much about the nature of viruses in hopes of one day controlling them, they also posed a clear threat in terms of creating harmful biochemical substances. At the time, the practical aspect of Lederberg's work was hard to evaluate. The Nobel Prize Committee, however recognized the significance of his contributions to genetics and, in 1958, awarded him the Nobel Prize in physiology or medicine for the bacterial and viral research that provided a new line of investigations of viral diseases and cancer. Lederberg shared the prize with Beadle and Tatum.

Lederberg's work in genetics eventually proved to be one of the foundations of gene mapping, which eventually led to efforts to genetically treat disease and identify those at risk of developing certain diseases.

Known as brilliant laboratory scientist and technician, Lederberg was also concerned with the role of science in society and the far-reaching effects of scientific discoveries, particularly in genetics. In a Pan American Health Organization/World Health Organization lecture in biomedical sciences called "Health in the World Tomorrow," Lederberg acknowledged concerns of the public, and even some scientists, over the newfound ability to tamper with the **genetic code** of life. However, he was more concerned with the many ethical questions that would arise over the inevitable success of the technological advances in microbiology and genetics. Lederberg saw the biological revolution as "a philosophical one" that was to bring a "new depth of scientific understanding about the nature of life." He foresaw advancements in the treatment of cancer, organ transplants, and geriatric medicine as presenting a whole new set of ethical and social problems, such as the availability and allocation of expensive health-care resources.

Lederberg was also interested in the study of biochemical life outside of Earth and coined the term *exobiology* to refer to such studies. Along with physicist Dean B. Cowie, he expressed concern in *Science* over the possible **contamination** of biological life on other planets from microbes carried by human spacecraft. He was also a consultant to the U.S. Viking space missions to the planet Mars.

Lederberg's career included an appointment as chairman of the new genetics department at Stanford University in 1958. In 1978 he was appointed president of Rockefeller University. Working with his first wife, Esther Zimmer, a former student of Tatum's whom Lederberg married in 1946, Lederberg investigated the role of bacterial **enzymes** in sugar **metabolism**. He also discovered that penicillin's ability to kill bacteria was due to its preventing synthesis of the bacteria's cell walls. Among Lederberg's many honors were the Eli Lilly Award for outstanding work by a scientist under thirty-five years of age and the Alexander Hamilton Medal of Columbia University.

See also Escherichia coli (E. coli); Microbial genetics; Molecular biology and molecular genetics; Viral genetics

LEEUWENHOEK, ANTONI VAN (1632-1723)

Dutch microscopist

Antoni van Leeuwenhoek is best remembered as the first person to study **bacteria** and "animalcules," or one-celled organisms now known as *protozoa*. Unlike his contemporaries **Robert Hooke** and Marcello Malpighi, Leeuwenhoek did not use the more advanced compound **microscope**; instead, he strove to manufacture magnifying lenses of unsurpassed power and clarity that would allow him to study the microcosm in far greater detail than any other scientist of his time.

Antoni van Leeuwenhoek, the "father" of microscopy, pictured with one of his light microscopes used to observe "animalcules."

Leeuwenhoek was born on October 24, 1632, in Delft, Holland. Although his family was relatively prosperous, he received little formal education. After completing grammar school in Delft, he moved to Amsterdam to work as a draper's apprentice. In 1654, he returned to Delft to establish his own shop, and he worked as a draper for the rest of his life. In addition to his business, Leeuwenhoek was appointed to several positions within the city government, which afforded him the financial security to spend a great deal of time and money in pursuit of his hobby, lens grinding. Lenses were important tools in Leeuwenhoek's profession, as cloth merchants often used small lenses to inspect their products. His hobby soon turned to obsession, however, as he searched for more and more powerful lenses.

In 1671, Leeuwenhoek constructed his first simple microscope. It consisted of a tiny lens that he had ground by hand from a globule of glass and placed within a brass holder. To this, he had attached a series of pins designed to hold the specimen. It was the first of nearly six hundred lenses ranging from 50 to 500 times magnifications that he would grind during his lifetime. Through his microscope, Leeuwenhoek examined such substances as skin, hair, and his own blood. He studied the structure of ivory as well as the physical composition of the flea, discovering that fleas, too, harbored **parasites**.

Leeuwenhoek began writing to the British Royal Society in 1673. At first, the Society gave his letters little

notice, thinking that such magnification from a single lens microscope could only be a hoax. However, in 1676, when he sent the Society the news that he had discovered tiny one-celled animals in rainwater, the interest of member scientists was piqued. Following Leeuwenhoek's specifications, they built microscopes of comparable magnitude and confirmed his findings. In 1680, the Society unanimously elected Leeuwenhoek as a member.

Until this time, Leeuwenhoek had been operating in an informational vacuum; he read only Dutch and, consequently, was unable to learn from the published works of Hooke and Malpighi (though he often gleaned what he could from the illustrations within their texts). As a member of the Society, he was finally able to interact with other scientists. In fact, the news of his discoveries spread worldwide, and he was often visited by royalty from England, Prussia, and Russia. The traffic through his laboratory was so persistent that he eventually allowed visitors by appointment only. Near the end of his life, Leeuwenhoek had reached near-legendary status and was often referred to by the local townsfolk as a magician.

Amid the attention, Leeuwenhoek remained focused upon his scientific research. Specifically, he was interested in disproving the common belief in spontaneous generation, a theory proposing that certain inanimate objects could generate life. For example, it was assumed that **mold** and maggots were created spontaneously from decaying food. Leeuwenhoek succeeded in disproving spontaneous generation in 1683, when he discovered bacteria cells. These tiny organisms were nearly beyond the resolving power of even Leeuwenhoek's remarkable equipment and would not be seen again for more than a century.

Leeuwenhoek created and improved upon new lenses for most of his long life. For the forty-three years that he was a member of the Royal Society, he wrote nearly 200 letters that described his progress. However, he never divulged the method by which he illuminated his specimens for viewing, and the nature of that illumination is still somewhat of a mystery. Upon his death on August 30, 1723, Leeuwenhoek willed twenty-six of his microscopes, a few of which survive in museums, to the British Royal Society.

See also Bacterial growth and division; Bacterial kingdoms; Bacterial membranes and cell wall; Bacterial movement; History of microbiology; Microscope and microscopy

LEGIONNAIRES' DISEASE

Legionnaires' disease is a type of **pneumonia** caused by *Legionella* **bacteria**. The bacterial species responsible for Legionnaires' disease is *L. pneumophila*. Major symptoms include fever, chills, muscle aches, and a cough that is initially nonproductive. Definitive diagnosis relies on specific laboratory tests for the bacteria, bacterial antigens, or antibodies produced by the body's **immune system**. As with other types of pneumonia, Legionnaires' disease poses the greatest threat to people who are elderly, ill, or immunocompromised.

Legionella bacteria were first identified as a cause of pneumonia in 1976, following an outbreak of pneumonia among people who had attended an American Legion convention in Philadelphia, Pennsylvania (the bacterium's name was derived from this group's name). This outbreak prompted further investigation into *Legionella* and it was discovered that earlier unexplained pneumonia outbreaks were linked to the bacteria. The earliest cases of Legionnaires' disease were shown to have occurred in 1965, but samples of the bacteria exist from 1947.

Exposure to the *Legionella* bacteria does not necessarily lead to infection. According to some studies, an estimated 5–10% of the American population show serologic evidence of exposure, the majority of whom do not develop symptoms of an infection. *Legionella* bacteria account for 2–15% of the total number of pneumonia cases requiring hospitalization in the United States.

There are at least 40 types of *Legionella* bacteria, half of which are capable of producing disease in humans. A disease that arises from infection by *Legionella* bacteria is referred to as legionellosis. The *L. pneumophila* bacterium, the root cause of Legionnaires' disease, causes 90% of legionellosis cases. The second most common cause of legionellosis is the *L. micdadei* bacterium, which produces the Philadelphia pneumonia-causing agent.

Approximately 10,000–40,000 people in the United States develop some type of Legionnaires' disease annually. The people who are the most likely to become ill are over age 50. The risk is greater for people who suffer from health conditions such as malignancy, diabetes, lung disease, or kidney disease. Other risk factors include immunosuppressive therapy and cigarette smoking. Legionnaires' disease has occurred in children, but typically, it has been confined to newborns receiving respiratory therapy, children who have had recent operations, and children who are immunosuppressed. People with **HIV** infection and **AIDS** do not seem to contract Legionnaires' disease with any greater frequency than the rest of the population, however, if contracted, the disease is likely to be more severe compared to other cases.

Cases of Legionnaires' disease that occur in conjunction with an outbreak, or epidemic, are more likely to be diagnosed quickly. Early diagnosis aids effective and successful treatment. During epidemic outbreaks, fatalities have ranged from 5% for previously healthy individuals to 24% for individuals with underlying illnesses. Sporadic cases (that is, cases unrelated to a wider outbreak) are harder to detect and treatment may be delayed pending an accurate diagnosis. The overall fatality rate for sporadic cases ranges from 10–19%. The outlook is bleaker in severe cases that require respiratory support or dialysis. In such cases, fatality may reach 67%.

Legionnaires' disease is caused by inhaling *Legionella* bacteria from the environment. Typically, the bacteria are dispersed in aerosols of contaminated water. These aerosols are produced by devices in which warm water can stagnate, such as air-conditioning cooling towers, humidifiers, shower heads, and faucets. There have also been cases linked to whirlpool spa baths and water misters in grocery store produce departments. Aspiration of contaminated water is also a potential

source of infection, particularly in hospital-acquired cases of Legionnaires' disease. There is no evidence of person-to-person transmission of Legionnaires' disease.

Once the bacteria are in the lungs, cellular representatives of the body's immune system (alveolar macrophages) congregate to destroy the invaders. The typical macrophage defense is to phagocytose the invader and demolish it in a process analogous to swallowing and digesting it. However, the *Legionella* bacteria survive being phagocytosed. Instead of being destroyed within the macrophage, they grow and replicate, eventually killing the macrophage. When the macrophage dies, many new *Legionella* bacteria are released into the lungs and worsen the infection.

Legionnaires' disease develops 2–10 days after exposure to the bacteria. Early symptoms include lethargy, headaches, fever, chills, muscle aches, and a lack of appetite. Respiratory symptoms such as coughing or congestion are usually absent. As the disease progresses, a dry, hacking cough develops and may become productive after a few days. In about a third of Legionnaires' disease cases, blood is present in the sputum. Half of the people who develop Legionnaires' disease suffer shortness of breath and a third complain of breathing-related chest pain. The fever can become quite high, reaching 104°F (40°C) in many cases, and may be accompanied by a decreased heart rate.

Although the pneumonia affects the lungs, Legionnaires' disease is accompanied by symptoms that affect other areas of the body. About half the victims experience diarrhea and a quarter have nausea and vomiting and abdominal pain. In about 10% of cases, acute renal failure and scanty urine production accompany the disease. Changes in mental status, such as disorientation, confusion, and hallucinations, also occur in about a quarter of cases.

In addition to Legionnaires' disease, *L. pneumophila* legionellosis also includes a milder disease, Pontiac fever. Unlike Legionnaires' disease, Pontiac fever does not involve the lower respiratory tract. The symptoms usually appear within 36 hours of exposure and include fever, headache, muscle aches, and lethargy. Symptoms last only a few days and medical intervention is usually not necessary.

The symptoms of Legionnaires' disease are common to many types of pneumonia and diagnosis of sporadic cases can be difficult. The symptoms and chest x rays that confirm a case of pneumonia are not useful in differentiating between Legionnaires' disease and other pneumonias. If a pneumonia case involves multisystem symptoms, such as diarrhea and vomiting, and an initially dry cough, laboratory tests are done to definitively identify *L. pneumophila* as the cause of the infection.

If Legionnaires' disease is suspected, several tests are available to reveal or indicate the presence of *L. pneumophila* bacteria in the body. Since the immune system creates antibodies against infectious agents, examining the blood for these indicators is a key test. The level of **immunoglobulins**, or **antibody** molecules, in the blood reveals the presence of infection. In microscopic examination of the patient's sputum, a fluorescent stain linked to antibodies against *L. pneumophila* can uncover the presence of the bacteria. Other means of revealing the bacteria's presence from patient sputum samples include isolation of the organism on **culture** media or detection of the bacteria by **DNA** probe. Another test detects *L. pneumophila* antigens in the urine.

The Bellevue-Stratford Hotel in Philadelphia, where an outbreak at a Legionnaires' convention gave the disease its name.

The type of antibiotic prescribed by the doctor depends on several factors including the severity of infection, potential **allergies**, and interaction with previously prescribed drugs. For example, erythromycin interacts with warfarin, a blood thinner. Several drugs, such as penicillins and cephalosporins, are normally ineffective against the infection. Although they may be deadly to the bacteria in laboratory tests, their chemical structure prevents them from being absorbed into the areas of the lung where the bacteria are present. In severe cases with complications, antibiotic therapy may be joined by respiratory support. If renal failure occurs, dialysis is required until renal function is recovered.

Appropriate medical treatment has a major impact on recovery from Legionnaires' disease. Outcome is also linked to the victim's general health and absence of complications. If the patient survives the infection, recovery from Legionnaires' disease is usually complete. Similar to other types of pneumo-

nia, severe cases of Legionnaires' disease may cause scarring in the lung tissue as a result of the infection. Renal failure, if it occurs, is reversible and renal function returns as the patient's health improves. Occasionally, fatigue and weakness may linger for several months after the infection has been successfully treated.

Because the bacteria thrive in warm stagnant water, regularly disinfecting ductwork, pipes, and other areas that may serve as breeding areas is the best method for preventing outbreaks of Legionnaires' disease. Most outbreaks of Legionnaires' disease can be traced to specific points of exposure, such as hospitals, hotels, and other places where people gather. Sporadic cases are harder to determine and there is insufficient evidence to point to exposure in individual homes.

See also Pneumonia, bacterial and viral

LEPROSY

Leprosy, also called Hansen's disease, affects 10 –12 million people worldwide. Caused by an unusual bacterium called *Mycobacterium leprae,* leprosy primarily affects humans. Leprosy is found in tropical areas, such as Africa, South and Southeast Asia, and Central and South America. In the United States, cases of leprosy have been reported in areas of Texas, California, Louisiana, Florida, and Hawaii. Leprosy can take many forms, but the most familiar form is characterized by skin lesions and nerve damage. Although leprosy is curable with various **antibiotics**, it remains a devastating illness because of its potential to cause deformity, especially in the facial features. Fortunately, antibiotic regimens are available to treat and eventually cure leprosy.

Mycobacterium leprae is an unusual bacterium for several reasons. The bacterium divides slowly; in some tests, researchers have noted a dividing time of once every twelve days. This differs from the dividing time of most **bacteria**, which is about once every few hours. *M. leprae* cannot be grown on **culture** media, and is notoriously difficult to culture within living animals. Because of these culturing difficulties, researchers have not been able to investigate these bacteria as closely as they have other, more easily cultured, bacteria. Questions remain unanswered about *M. leprae;* for instance, researchers are still unclear about how the bacteria are transmitted from one person to another, and are not sure about the role an individual's genetic make up plays in the progression of the disease.

Because *M. leprae* almost exclusively infects humans, animal models for studying leprosy are few. Surprisingly, a few species of armadillo can also be infected with *M. leprae*. Recently, however, wild armadillos have been appearing with a naturally occurring form of leprosy. If the disease spreads in the armadillo population, researchers will not be able to use these animals for leprosy studies, since study animals must be completely free of the disease as well as the bacteria that cause it. Mice have also been used to study leprosy, but laboratory conditions, such as temperature, must be carefully controlled in order to sustain the infection in mice.

M. leprae is temperature-sensitive; it favors temperatures slightly below normal human body temperature. Because of this predilection, *M. leprae* infects superficial body tissues such as the skin, bones, and cartilage, and does not usually penetrate to deeper organs and tissues. *M. leprae* is an intracellular pathogen; it crosses host cell membranes and lives within these cells. Once inside the host cell, the bacterium reproduces. The time required by these slow-growing bacteria to reproduce themselves inside host cells can be anywhere from a few weeks to as much as 40 years. Eventually, the bacteria lyse (burst open) the host cell, and new bacteria are released that can infect other host cells.

Researchers assume that the bacteria are transmitted via the respiratory tract. *M. leprae* exists in the nasal secretions and in the material secreted by skin lesions of infected individuals. *M. leprae* has also been found in the breast milk of infected nursing mothers. *M. leprae* may be transmitted by breathing in the bacteria, through breaks in the skin, or perhaps through breast-feeding.

Leprosy exists in several different forms, although the infectious agent for all of these forms is *M. leprae*. Host factors such as genetic make up, individual **immunity**, geography, ethnicity, and socioeconomic circumstances determine which form of leprosy is contracted by a person exposed to *M. leprae*. Interestingly, most people who come in contact with the bacterium, about three-fourths, never develop leprosy, or develop only a small lesion on the trunk or extremity that heals spontaneously. Most people, then, are not susceptible to *M. leprae,* and their immune systems function effectively to neutralize the bacteria. But one-fourth of those exposed to *M. leprae* contract the disease, which may manifest itself in various ways.

Five forms of leprosy are recognized, and a person may progress from one form to another. The least serious form is tuberculoid leprosy. In this form, the skin lesions and nerve damage are minor. Tuberculoid leprosy is evidence that the body's cellular immune response—the part of the **immune system** that seeks out and destroys infected cells—is working at a high level of efficiency. Tuberculoid leprosy is easily cured with antibiotics.

If tuberculoid leprosy is not treated promptly, or if a person has a less vigorous cellular immune response to the *M. leprae* bacteria, the disease may progress to a borderline leprosy, which is characterized by more numerous skins lesions and more serious nerve damage. The most severe form of leprosy is lepromatous leprosy. In this form of leprosy, the skin lesions are numerous and cause the skin to fold, especially the skin on the face. This folding of facial skin leads to the leonine (lion-like) features typical of lepromatous leprosy. Nerve damage is extensive, and people with lepromatous leprosy may lose the feeling in their extremities, such as the fingers and toes. Contrary to popular belief, the fingers and toes of people with this form of leprosy do not spontaneously drop off. Rather, because patients cannot feel pain because of nerve damage, the extremities can become easily injured.

Lepromatous leprosy occurs in people who exhibit an efficient **antibody** response to *M. leprae* but an inefficient cellular immune response. The antibody arm of the immune system is not useful in neutralizing intracellular pathogens such as

The disfiguring effect of leprosy.

M. leprae; therefore, people who initially react to invasion by M. leprae by making antibodies may be at risk for developing more severe forms of leprosy. Researchers are not sure what determines whether a person will react with a cellular response or an antibody response; current evidence suggests that the cellular immune response may be controlled by a special **gene**. If a person has this gene, he or she will probably develop the less severe tuberculoid leprosy if exposed to *M. leprae*.

Treatments for leprosy have improved considerably over the past 40 years. In fact, some experts are encouraged that the drug regimens being tested in various trials throughout the world (including the United States) may eradicate leprosy completely by the year 2010. Beginning in the 1950s, an antibiotic called dapsone was used to treat leprosy, offering the first hope of a cure for persons with the disease. Dapsone's main disadvantage was that the patient had to take the medication daily throughout his or her lifetime. In addition, the *M. leprae* in some patients underwent genetic **mutations** that rendered it resistant to the antibiotic. In the 1960s, the problem of resistance was tackled with the advent of multidrug therapy. Bacteria are less likely to become resistant to several drugs given in combination. The new multidrug treatment time was also considerably shorter-typically about four years. Currently, researchers offer a new drug combination that includes an antibiotic called oflaxicin. Oflaxicin is a powerful inhibitor of certain bacterial **enzymes** that are involved in **DNA** coiling. Without these enzymes, the *M. leprae* cannot copy the DNA properly and the bacteria die. The treatment time for this current regimen is about four weeks or less, the shortest treatment duration so far.

One risk of treatment, however, is that antigens—the proteins on the surface of *M. leprae* that initiate the host immune response—are released from the dying bacteria. In some people, when the antigens combine with antibodies to *M. leprae* in the bloodstream, a reaction called erythema nodosum leprosum may occur, resulting in new lesions and peripheral nerve damage. In the late 1990s, the drug thalidomide was approved to treat this reaction, with good results. Because thalidomide may cause severe birth defects, women of childbearing age must be carefully monitored while taking the drug.

A promising development in the treatment and management of leprosy is the preliminary success shown by two different vaccines. One **vaccine** tested in Venezuela combined a vaccine originally developed against **tuberculosis**, called Bacille Calmette-Guerin (BCG), and heat-killed *M. leprae* cultured from infected armadillos. The other vaccine uses a relative of *M. leprae* called *M. avium*. The advantage of this vaccine, currently being tested in India, is that *M. avium* is easy to culture on media and is thus cheaper than the Venezuelan vaccine. Both vaccines have performed well in their clinical trials, leading many to hope that a vaccine against leprosy might soon be available.

The **World Health Organization** announced in January, 2002, that during the previous decade, the number of active cases of leprosy worldwide had been reduced by 90%. Control of leprosy still eludes six countries, Brazil, India, Madagascar, Mozambique, Myanmar and Nepal, with approximately 700,

000 ongoing cases identified worldwide in 2002. In conjunction with the World Health Organization, these countries have committed to accelerating control efforts, including early access to current drug therapy. The worldwide reduction and control of leprosy stands as one of the major world health initiatives of modern times.

See also Bacteria and bacterial infection; Mycobacterial infections, atypical

LETHAL DOSE, LD50 • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

LICHEN PLANUS

Lichen planus is a skin rash characterized by small, flat-topped, itchy purplish raised spots on the wrists, arms, or lower legs. Although the evidence is not conclusive, many researchers assert that Lichen planus is an autoimmune disease.

Lichen planus affects approximately one to two percent of the population. Although there is no apparent correlation to race or geographic region, it is interesting to note that the majority of individuals affected are women, age 30 to 50 years. Lichen planus rashes may produce discoloration of the skin, especially in darker skinned population groups. Lichen planus lesions may develop on the genitals or in the mouth. Within a few years, most of the spots disappear, even without treatment.

Although not definitive, researchers assert Lichen planus exhibits many of the characteristics of an autoimmune disorder. Autoimmune diseases result when the **immune system** attacks the body's own cells, causing tissue destruction. Dermatologists argue that the condition may result from a viral infection that is then aggravated by stress. Lichen planus symptoms are similar to allergic reactions to arsenic, gold, and bismuth. The spots are also similar to the type produced from allergic reactions to certain chemicals used to develop film.

There is a correlation (a statistical relationship) between allergic reactions to certain medications and the appearance of a Lichen planus rash in the mouth. Oral lichen planus usually forms white lines and spots that may appear in clusters. Only a definitive biopsy can fully distinguish the rash from **yeast** infections or canker sores. Dentists find that some patients develop a Lichen planus rash following dental procedures. Other reports indicate that a Lichen planus rash may appear as an allergic-reaction like response to certain foods, candy, or chewing gum.

Because the exact cause of Lichen planus is unknown, there is no specific treatment for the rash. Treatment with various combinations of steroid creams, oral corticosteroids, and oral antihistamines appears effective at relieving discomfort caused by the rash. In more severe cases PUVA photochemotherapy, a procedure where cells are photosensitizing and then exposed to ultraviolet light and **antibiotics**.

Lichen planus may also affect the growth of nails and, if present on the scalp, may contribute to hair loss.

Lichen planus is not an infectious disease. Research also indicates that it is not, as once argued, caused by any specific dietary deficiency.

See also Autoimmunity and autoimmune diseases; Viruses and responses to viral infection; Yeast, infectious

LICHENS

Lichens are an intimate symbiosis, in which two species live together as a type of composite organism. Lichens are an obligate mutualism between a fungus mycobiont and an alga or blue-green bacterium phycobiont.

Each lichen mutualism is highly distinctive, and can be identified on the basis of its size, shape, color, and **biochemistry**. Even though lichens are not true "species" in the conventional meaning of the word, lichenologists have developed systematic and taxonomic treatments of these mutualisms.

The fungal partner in the lichen mutualism gains important benefits through access to photosynthetic products of the alga or blue-green bacterium. The phycobiont profits from the availability of a relatively moist and protected habitat, and greater access to inorganic nutrients.

The most common **fungi** in lichens are usually species of *Ascomycetes,* or a few *Basidiomycetes*. The usual algal partners are either species of green algae **Chlorophyta** or blue-green **bacteria** of the family Cyanophyceae. In general, the fungal partner cannot live without its phycobiont, but the algae is often capable of living freely in moist soil or water. The largest lichens can form a thallus up to 3 ft (1 m) long, although most lichens are smaller than a few inches or centimeters in length. Lichens can be very colorful, ranging from bright reds and oranges, to yellows and greens, and white, gray, and black hues.

Most lichens grow very slowly. Lichens in which the phycobiont is a blue-green bacterium have the ability to fix nitrogen gas into ammonia. Some lichens can commonly reach ages of many centuries, especially species living in highly stressful environments, such as alpine or arctic tundra.

Lichens can grow on diverse types of substrates. Some species grow directly on rocks, some on bare soil, and others on the bark of tree trunks and branches. Lichens often grow under exposed conditions that are frequently subjected to periods of drought, and sometimes to extremes of hot and cold. Lichen species vary greatly in their tolerance of severe environmental conditions. Lichens generally respond to environmental extremes by becoming dormant, and then quickly becoming metabolically active again when they experience more benign conditions.

Lichens are customarily divided into three growth forms, although this taxonomy is one of convenience, and is not ultimately founded on systematic relationships. Crustose lichens form a thallus that is closely appressed to the surface upon which they are growing. Foliose lichens are only joined to their substrate by a portion of their thallus, and they are somewhat

leaf-like in appearance. Fruticose lichens rise above their substrate, and are much branched and bushy in appearance.

Most lichens regenerate asexually as lichen symbioses, and not by separate reproduction of their mycobiont and phycobiont. Reproduction is most commonly accomplished by small, specialized fragments of thallus known as soredia, consisting of fungal tissue enclosing a small number of algal cells. The soredia generally originate within the parent thallus, then grow out through the surface of the thallus, and detach as small bits of tissue that are dispersed by the wind or rain. If the dispersing soredium is fortunate enough to lodge in a favorable microenvironment, it develops into a new thallus, genetically identical to the parent.

Because they are capable of colonizing bare rocks and other mineral substrates, lichens are important in soil formation during some ecological successions. For example, lichens are among the first organisms to colonize sites as they are released from glacial ice. In such situations, lichens can be important in the initial stages of nitrogen accumulation and soil development during post-glacial primary succession.

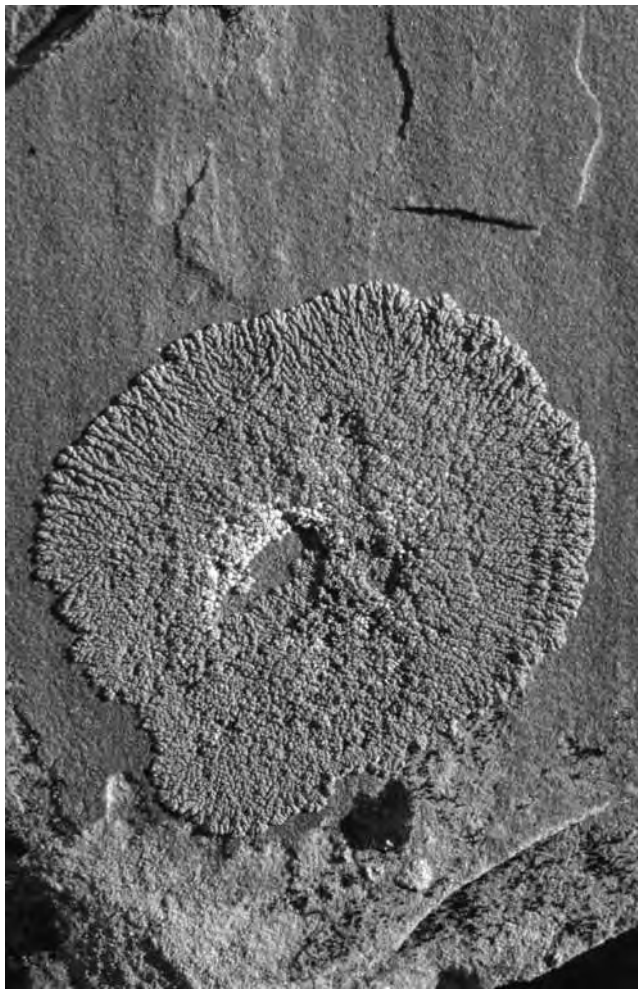
Lichens are important forage for some species of animals. The best known example of this relationship involves the northern species of deer known as caribou or reindeer (*Rangifer tarandus*) and the so-called reindeer lichens (*Cladina spp.*) that are one of their most important foods, especially during winter.

Some species of lichens are very sensitive to air pollutants. Consequently, urban environments are often highly impoverished in lichen species. Some ecologists have developed schemes by which the intensity of air pollution can be reliably assayed or monitored using the biological responses of lichens in their communities. Monitoring of air quality using lichens can be based on the health and productivity of these organisms in places variously stressed by toxic pollution. Alternatively, the chemical composition of lichens may be assayed, because their tissues can effectively take up and retain sulfur and metals from the atmosphere.

Some lichens are useful as a source of natural dyes. Pigments of some of the more colorful lichens, especially the orange, red, and brown ones, can be extracted by boiling and used to dye wool and other fibers. Other chemicals extracted from lichens include litmus, which was a commonly used acid-base indicator prior to the invention of the **pH** meter.

Some of the reindeer lichens, especially *Cladina alpestris,* are shaped like miniature shrubs and trees. Consequently, these plants are sometimes collected, dried, and dyed, and are used in "landscaping" the layouts for miniature railroads and architectural models.

In addition, lichens add significantly to the aesthetics of the ecosystems in which they occur. The lovely orange and yellow colors of *Caloplaca* and *Xanthoria* lichens add much to the ambience of rocky seashores and tundras. The intricate webs of filamentous *Usnea* lichens hanging in profusion from tree branches give a mysterious aspect to humid forests. These and other, less charismatic lichens are integral components of their natural ecosystems. These lichens are intrinsically important for this reason, as well as for the relatively minor benefits that they provide to humans.



A lichen growing on a rock.

LIFE, ORIGIN OF

The origin of life has been a subject of speculation in all known cultures and indeed, all have some sort of creation idea that rationalizes how life arose. In the modern era, this question has been considered in terms of a scientific framework, meaning that it is approached in a manner subject to experimental verification as far as that is possible. Radioactive dating suggests that Earth formed at least 4.6 billion years ago. Yet, the earliest known fossils of **microorganisms**, similar to modern **bacteria**, are present in rocks that are 3.5–3.8 billion years old. The earlier prebiotic era (i.e., before life began) left no direct record, and so it cannot be determined exactly how life arose. It is possible, however, to at least demonstrate the kinds of abiotic reactions that may have led to the formation of living systems through laboratory experimentation. It is generally accepted that the development of life occupied three stages: First, chemical **evolution**, in which simple geologically occurring molecules reacted to form complex organic polymers. Second, collections of these polymers self organized to form replicating entities. At some

The sea.

point in this process, the transition from a lifeless collection of reacting molecules to a living system probably occurred. The third process following organization into simple living systems was biological evolution, which ultimately produced the complex web of modern life.

The underlying biochemical and genetic unity of organisms suggests that life arose only once, or if it arose more than once, the other life forms must have become rapidly extinct. All organisms are made of chemicals rich in the same kinds of carbon-containing, organic compounds. The predominance of carbon in living matter is a result of its tremendous chemical versatility compared with all the other elements. Carbon has the unique ability to form a very large number of compounds as a result of its capacity to make as many as four highly stable covalent bonds (including single, double, triple bonds) combined with its ability to form covalently linked C—C chains of unlimited length. The same 20 carbon and nitrogen containing compounds called amino acids combine to make up the enormous diversity of proteins occurring in living things. Moreover, all organisms have their genetic blueprint encoded in nucleic acids, either **DNA** or **RNA**. Nucleic acids contain the information needed to synthesize specific proteins from their amino acid components. **Enzymes**, catalytic proteins, which increase the speed of specific chemical reactions, regulate the activity of nucleic acids and other biochemical functions essential to life, while other proteins provide the structural framework of cells. These two types of molecules, nucleic acids and proteins, are essential enough to all organisms that they, or closely related compounds, must also have been present in the first life forms.

Scientists suspect that the primordial Earth's atmosphere was very different from what it is today. The modern atmosphere with its 79% nitrogen, 20% oxygen, and trace quantities of other gases is an oxidizing atmosphere. The primordial atmosphere is generally believed not to have contained significant quantities of oxygen, having instead rather small amounts of gases such as carbon monoxide, methane, ammonia and sulphate in addition to the water, nitrogen and carbon dioxide, which it still contains today. With these combinations of gases, the atmosphere at that time would have been a reducing atmosphere providing the hydrogen atoms for the synthesis of compounds needed to create life. In the 1920s, the Soviet scientist Aleksander Oparin (1894–1980) and the British scientist J.B.S. Haldane (1892–1964) independently suggested that ultraviolet (UV) light, which today is largely absorbed by the ozone layer in the higher atmosphere, or violent lightning discharges, caused molecules of the primordial reducing atmosphere to react and form simple organic com-

pounds (e.g., amino acids, nucleic acids and sugars). The possibility of such a process was demonstrated in 1953 by Stanley Miller and **Harold Urey**, who simulated the effects of lightning storms in a primordial atmosphere by subjecting a refluxing mixture of water, methane, ammonia and hydrogen to an electric discharge for about a week. The resulting solution contained significant amounts of water-soluble organic compounds including amino acids.

The American scientist, Norman H. Horowitz proposed several criteria for living systems, saying that they all must exhibit replication, catalysis and mutability. One of the chief features of living organisms is their ability to replicate. The primordial self-replicating systems are widely believed to have been nucleic acids, like DNA and RNA, because they could direct the synthesis of molecules complementary to themselves. One hypothesis for the evolution of self-replicating systems is that they initially consisted entirely of RNA. This idea is based on the observation that certain species of ribosomal RNA exhibit enzyme-like catalytic properties, and that all nucleic acids are prone to mutation. Thus, RNA can demonstrate the three Horowitz criteria and the primordial world may well have been an "RNA world". A cooperative relationship between RNA and protein could have arisen when these self-replicating protoribosomes evolved the ability to influence the synthesis of proteins that increased the efficiency and accuracy of RNA synthesis. All these ideas suggest that RNA was the primary substance of life and the later participation of DNA and proteins were later refinements that increased the survival potential of an already self-replicating living system. Such a primordial pond where all these reactions were evolving eventually generated compartmentalization amongst its components. How such cell boundaries formed is not known, though one plausible theory holds that membranes first arose as empty vesicles whose exteriors served as attachment sites for entities such as enzymes and **chromosomes** in ways that facilitated their function.

See also DNA (Deoxyribonucleic acid); Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Miller-Urey experiment; Ribonucleic acid (RNA)

LIGHT MICROSCOPY • *see* MICROSCOPE AND MICROSCOPY

LIPOPOLYSACCHARIDE AND ITS CONSTITUENTS

Lipopolysaccharide (LPS) is a molecule that is a constituent of the outer membrane of Gram-negative **bacteria**. The molecule can also be referred to as endotoxin. LPS can help protect the bacterium from host defenses and can contribute to illness in the host.

The LPS comprises much of the portion of the outer membrane that is oriented towards the outside of the bacterium. There are fewer phospholipid molecules in this outer "leaflet" of the membrane than there are on the inner side of the membrane. Thus, because of the presence of lipopolysaccahride, the construction of the outer membrane is asymmetric. In contrast, the inner membrane of Gram-negative bacteria and the single membrane of Gram-positive bacteria are more symmetric, with both leaflets of the membrane comprised of much the same molecules.

A complete LPS consists of a lipid portion and a chain of sugar. The lipid region is anchored into the inner portion of the membrane by a molecule called lipid A. A core polysaccharide is also considered part of the lipid region. This core contains a compound known as 2-keto-3-deoxyoctonic acid, or KDO. The lipid A and KDO portions of LPS are common to all bacterial LPS.

The other region of LPS is the sugar chain. This portion is also known as the O-antigen. The O-antigen gets its name from the fact that it is exposed to the external environment and will be the target of **antibody formation** by the host. The hydrophilic ("water-loving") sugar side chain extends outward from the surface of the cell into the watery environment that typically surrounds many Gram-negative bacteria. There are many chemical arrangements of the sugar chain.

The manufacture of LPS is a multi-step process involving many **enzymes**. The complete LPS molecule is incorporated into the outer membrane. The biosynthetic pathway of LPS was deduced by the isolation of **mutants** defective in LPS assembly.

LPS can be detected using microscopic techniques following the binding of LPS specific **antibody**. Additionally, a biochemical test can be done. The test utilizes a compound that is obtained from the horseshoe crab.

Not all bacteria have a complete sugar chain. Depending on the bacterial species a portion of the sugar chain can be present, or sugar chain may be entirely absent. The various LPS chemistries have an affect on the appearance of the bacteria when they are grown as colonies on solid growth media. Those bacteria with the complete side chain can appear smooth and even wet, whereas those with no side chain often appear crinkly and dry. For this reason, bacteria having the complete LPS are known as smooth strains and those bacteria with no sugar side chain are designated as rough strains. Those species of bacteria that are in between, having a portion of the sugar side chain, are called semi-rough strains.

The composition of the LPS also affects the overall chemistry of the bacterial surface. Because the sugar chains protruding from the surface are hydrophilic, the bacterium tends to prefer watery environments. In contrast the lack of the side chains exposes the **hydrophobic** ("water hating") lipid portion of the LPS. The surface of such bacteria tends to be more hydrophobic. In solution, the rough bacteria tend to clump together in an effort to avoid the water. Antibacterial compounds that are hydrophobic are more likely to penetrate into rough strains than into smooth strains. In the intestinal tract of warm-blooded animals, where many Gram-negative bacteria live, the possession of a complete LPS is advantageous for the absorption of hydrophilic nutrients by the bacteria.

The LPS structure has a profound influence on the potential of infectious Gram-negative bacteria to establish an

infection in humans and other animals. The sugar chains of smooth LPS can overlay the surface proteins of the outer membrane, masking the proteins from immune detection. Also, a bacterium can vary the chemistry of the O-antigen, so as to make the targeting of antibodies to the bacterial surface even more difficult. In contrast, the immune response to a rough strain, where the surface proteins are not camouflaged, is greater and more consistent.

Lipopolysaccharide is medically important to humans. When free from the bacterium, LPS is toxic. The portion of the LPS that is responsible for the toxicity is the core and lipid A portion (the endotoxin). Endotoxin can produce a fever, decrease in the number of white blood cells, and damage to blood vessels resulting in reduced blood pressure. At high enough endotoxin concentrations, shock can set in and death can occur.

See also Bacterial membranes and cell wall; Enterotoxin and exotoxin; Immunization

LIQUID MEDIA • *see* GROWTH AND GROWTH MEDIA

LISTER, JOSEPH (1827-1912)

English surgeon

Joseph Lister contributed to a fundamental revolution in surgery with the introduction of his antiseptic method. At the time Lister was practicing medicine, the mortality rate for certain injuries and surgeries was extremely high due to infection. The mortality rate dropped drastically with the use of an antiseptic method, and when used in conjunction with the anesthetics that were available at the time, surgeons dared to perform more complicated surgical procedures.

Lister was born to a well-known Quaker family at Upton, England. Lister studied medicine at University College, and received his medical degree in 1852. As a student, Lister had the opportunity to be a spectator at the first surgery performed with general anesthesia, performed by Robert Liston (1794–1847). He also studied histology under William Sharpey during which time, Lister wrote an important paper on **inflammation** where he discussed the susceptibility to disease of inflamed tissue. Lister was also interested in microscopic anatomy and physiology, perhaps because his father, Joseph Jackson Lister, was a microscopist. At one point, Lister wanted to become a surgeon and left England to study at Edinburgh University with James Syme (1799–1870), who was well known for his success with performing amputations and joint excisions. Syme, the first surgeon to adopt antisepsis and anesthesia, eventually became Lister's father-in-law.

As a surgeon, Lister was concerned with the high mortality rate of post-amputation patients and the high rate of gangrene after surgery. Applying the knowledge that **bacteria** caused disease, and drawing from Louis Pasteur's work that proved the existence of airborne **microorganisms**, Lister concluded that airborne bacteria could cause infection in surgical wounds. Lister read about the affect of carbolic acid used on sewage bacteria in outhouses, cesspools, and stables in the nearby town of Carlisle, and developed an antiseptic system whereby he would spray carbolic acid in the operating room, and use it to sterilize the surgical instruments and his hands. In addition, he applied the acid in and around the wound, and directly on the dressings. Lister first used this method in 1865 while treating a compound fracture of a leg, an injury that often claimed about 60% of patients, and where amputation of a limb was usually the only treatment. The procedure was successful. Lister published his antiseptic method in *The Lancet,* in 1867. There was one problem: carbolic acid, especially the spray, was harmful to those who came in contact with it. However, Lister found milder **antiseptics** and later heat-sterilized the surgical instruments. At first, the medical community did not support Lister's theory, but eventually his antiseptic method gained recognition and was adopted as standard procedure for treating wounds and during surgery. Medics used Lister's antiseptic method, which proved to be effective, during the Franco-Prussian War (1870–1871). In 1877, Lister became Professor of Surgery at King's College, London.

Lister received many honors and awards. A dedicated surgeon, he treated both inflicted and surgical wounds; he experimented with various antiseptics, developed absorbable sutures, and introduced a method of draining wounds. He was the first British surgeon to be elevated to the peerage (became a member of the House of Lords), and upon his death in 1912, his remains were interred in Westminster Abbey. When he died, it was said that Lister had saved more lives than all the wars in history had claimed.

See also Bacteria and bacterial infection; History of microbiology; History of public health; Infection and resistance; Infection control

LOEFFLER, FRIEDRICH AUGUST JOHANNES (1852-1915)

German physician

Friedrich August Loeffler was a German physician who turned his career path to focus on microbiology after becoming an assistant to **Robert Koch**. Loeffler is accredited with the discovery of several **microorganisms** including *Loefflerella mallei,* the etiological agent of glanders, *Corynebacterium diphtheriae,* the infectious organism of **diphtheria**; and *Erysipelothrix rhusiopathiae,* the infectious agent that causes cholera in swine. In addition to his discoveries of **bacteria**, Loeffler determined that **foot-and-mouth disease** was due to an infectious microorganism smaller than any bacteria (a virus).

Friedrich Loeffler began his studies in medicine at the University of Würzburg but then transferred to the Army Medical School shortly before the Franco-Prussian War. In 1872, Loeffler received his medical degree and then worked as an assistant physician in Berlin at the Charté Hospital. Beginning in 1876, he worked as a **public health** officer and military surgeon in Potsdam and Hannover. This lasted until

Joseph Lister (right) pioneered the use of antibacterial techniques in hospitals, including the use of disinfectant spray during operations.

1879, when he moved to Berlin and continued his work at the Kaiserliches Gesundheitsamt.

Friedrich Loeffler's transfer brought him under the supervision of Robert Koch. Loeffler and Georg Graffky began assisting Koch on his research of bacteria. Loeffler first began his bacteriological studies researching effective methods of **disinfection**. During his studies, Loeffler discovered *Loefflerella mallei,* bacteria that causes glanders, a disease seen mainly in horses. To determine the exact bacteria that causes glanders, Loeffler applied what has come to be known as **Koch's postulates**. Initially, Loeffler isolated the infectious agent from the horse and grew it in a pure **culture** of blood serum. Next, Loeffler injected the bacteria into healthy horses, which then showed symptoms of the disease. Finally, Loeffler once again isolated the bacteria from the once healthy horses. In addition to discovering *Loefflerella mallei,* Loeffler discovered the infectious agent that causes cholera in swine.

In 1884, after a long struggle to decipher the etiological agent that causes diphtheria, Loeffler isolated *Corynebacterium diphtheriae* in pure culture from the throat of humans. Problems pinpointing the exact microorganism that causes diphtheria stemmed from the fact that many different microorganisms inhabited the throats of diphtheria patients. Loeffler undertook the task of isolating pure cultures of bacteria to determine the exact etiological agent of diphtheria. Loeffler found that certain throat infections were due to streptococcal infections, which are now known to cause scarlet fever. He reasoned that the *Streptococcus* bacteria were not responsible for causing diphtheria because when injected into healthy animals, the bacteria did not produce symptoms characteristic of the disease. Additionally, the *Streptococcus* was not always abundant in diphtheria patients. The *Streptococcus* appeared to be secondary to rod shaped bacteria. When these rod shaped bacteria, called bacillus, were isolated in pure culture and injected into healthy animals, the animals exhibited the characteristic signs of human diphtheria, including the pseudomembrane in the throat of the patients that suffocate to death. Additionally, Loeffler determined that toxins produced by the infectious agent were the cause of destruction to internal organs. He reasoned that the toxins released entered the blood stream and traveled to other organs thereby poisoning them. Emile Roux and Yersin proved this theory of toxins to be correct. Loeffler made a surprising discovery when he was able to isolate the diphtheria bacillus from healthy individuals. He then determined that not all people who carry infectious microorganisms contract the disease.

Also in 1884, Loeffler began his new career as hygienic director with his first directorship position being at the First Garrison Hospital. There he was able to continue his bacteriological research in conjunction with lecturing on sanitation. Two years later, he became part of the faculty at the University

of Berlin. By 1888, he had transferred to the University of Greifswald where he spent the next 25 years.

At the University of Greifswald, Loeffler studied *Salmonella typhi-murium,* the bacteriological agent that causes mouse typhoid but does not infect other animals. This research was intended to control the exuberant mouse population that was threatening to destroy the crops of Greece. Loeffler effectively killed the mice by contaminating their food sources with the bacteria.

In 1898, Friedrich Loeffler, in conjunction with Paul Frosch, determined a filterable agent proving smaller than any bacteria previously discovered caused foot-and-mouth disease. This was the first hint that **viruses** existed. At that time, Loeffler was working at the University of Greifswald as head of the department of **hygiene**. Loeffler moved his laboratory to the island of the Insel Riems in order to safely continue his research on the disease. In 1913, Loeffler's research took a back seat to his new position as director of the Robert Koch Institute in Berlin. Once World War I began, all research on the Insel Riems ceased. Loeffler worked for the army to implement proper hygiene regimens until his death in 1915.

See also Coryneform bacteria; Streptococci and streptococcal infections

LUMINESCENT BACTERIA

Luminescence is the emission of light by an object. Living organisms including certain **bacteria** are capable of luminescence (**bioluminescence**). Bacteria are the most abundant luminescent organism in nature.

Bacterial luminescence has been studied most extensively in several marine bacteria (e.g., *Vibrio harveyi*, *Vibrio fischeri*, *Photobacterium phosphoreum*, *Photobacterium leiognathi*), and in *Xenorhabdus luminescens*, a bacteria that lives on land. The precise molecular mechanisms of luminescence differ between these bacteria. But, the general scheme of the process is similar.

In luminescent bacteria (and other luminescent organisms as well) this general scheme involves an enzyme that is dubbed luciferase. A suite of genes dubbed lux genes code for the enzyme and other components of the luminescent system. The different bacteria are dissimilar in the sequence of their lux genes and in the enzyme reactions that produce luminescence. However, the general pattern of the reaction is the same.

A similarity between the luminescent bacteria concerns the conditions that prompt the luminescence. A key factor is the number of bacteria that are present. This is also known as the cell density (i.e., the number of bacteria per given volume of solution or given weight of sample). A low cell density (e.g., less than 100 living bacteria per milliliter) does not induce luminescence, whereas luminescence is induced at a high cell density (e.g., 10^{10} to 10^{11} living bacteria per milliliter).

The effect of cell density is particularly evident in those luminescent bacteria that live in the ocean. When living free in the ocean water, *Vibrio fischeri* is not luminescent. However, when living in a confined space such as the inside of a fish or squid, *Vibrio fischeri* is luminescent. Bacterial luminescence may have evolved as a means of enhancing the survival of the bacteria species. For example, the luminescence of *Vibrio fischeri* in a squid enables the squid to camouflage itself from undersea predators in the moonlit ocean. In return for this protection, the squid provides the bacteria with a hospitable environment.

The influence of cell density on bacterial luminescence is due to the nature of the luminescent process. The bacteria produce a chemical called homoserine lactone. At low cell densities, the chemical exits a bacterium and drifts away in the fluid that surround the cell. But at high cell densities when the bacteria are tightly packed together, the homoserine lactone stays in the immediate vicinity of the bacteria. Then, the chemical is able to stimulate the activity of the lux genes that are responsible for the luminescence. This occurs when the homoserine lactone binds to a protein in the bacterial **cytoplasm** called LuxR. The LuxR-homoserine lactone complex then binds to a region of the bacterial **DNA** that is the master control for the activity of the lux genes.

Bacterial luminescence is due to the action of the enzyme called luciferase. Luciferase catalyses the removal of an electron from two compounds. Excess energy is liberated in this process. The energy is dissipated as a luminescent blue-green light. Luminescent bacteria contain a number of genes that are found linked to each other in the bacterial genome, and which are controlled by a common regulatory region of the DNA. This arrangement of genes is called an **operon**.

The lux genes are involved in the production of luciferase, in the production and activity of the LuxR protein that detects the homoserine lactone, and in the chemicals reactions that produce the compounds on which the luciferase acts.

Bacteria utilize homoserine lactone in other cell-to-cell communications that are cell-density dependent. One example is the formation of the adherent, exopolysaccharide-enmeshed populations, known as biofilms, by the bacterium *Pseudomonas aeruginosa*. Another example is the bacterium *Agrobacterium* that produces diseases in some plants. The phenomenon has been termed **quorum sensing**.

The lux **gene** system responsible for bacterial luminescence has become an important research tool and commercial product. The incorporation of the luminescent genes into other bacteria allows the development of bacterial populations to be traced visually. Because luminescence can occur over and over again and because a bacterium's cycle of luminescence is very short (i.e., a cell is essentially blinking on and off), luminescence allows a near instantaneous (i.e., "real time") monitoring of bacterial behavior. The use of lux genes is being extended to eukaryotic cells. This development has created the potential for the use of luminescence to study eukaryotic cell density related conditions such as cancer.

See also Bacterial adaptation; Bioluminescence; Economic uses and benefits of microorganisms

LYME DISEASE

Lyme disease is an infection transmitted by the bite of ticks carrying the spiral-shaped bacterium *Borrelia burgdorferi*. The disease was named for Lyme, Connecticut, the town where it was first diagnosed in 1975, after a puzzling outbreak of arthritis. The organism was named for its discoverer, Willy Burgdorfer. The effects of this disease can be long-term and disabling unless it is recognized and treated properly with **antibiotics**.

Lyme disease is a vector-borne disease, which means it is delivered from one host to another. In this case, a tick bearing the *B. burgdorferi* organism literally inserts it into a host's bloodstream when it bites the host to feed on its blood. It is important to note that neither *B. burgdorferi* nor Lyme disease can be transmitted from one person to another.

In the United States, Lyme disease accounts for more than 90% of all reported vector-borne illnesses. It is a significant **public health** problem and continues to be diagnosed in increasing numbers. More than 99,000 cases were reported between 1982 and 1996. When the numbers for 1996 Lyme disease cases reported were tallied, there were 16,455 new cases, a record high following a drop in reported cases from 1994 (13,043 cases) to 1995 (11,700 cases). Controversy clouds the true incidence of Lyme disease because no test is definitively diagnostic for the disease, and the broad spectrum of Lyme disease's symptoms mimic those of so many other diseases. Originally, public health specialists thought Lyme disease was limited geographically in the United States to the East Coast. Now it is known that it occurs in most states, with the highest number of cases in the eastern third of the country.

The risk for acquiring Lyme disease varies, depending on what stage in its life cycle a tick has reached. A tick passes through three stages of development—larva, nymph, and adult—each of which is dependent on a live host for food. In the United States, *B. burgdorferi* is borne by ticks of several species in the genus *Ixodes,* which usually feed on the white-footed mouse and deer (and are often called deer ticks). In the summer, the larval ticks hatch from eggs laid in the ground and feed by attaching themselves to small animals and birds. At this stage, they are not a problem for humans. It is the next stage, the nymph, that causes most cases of Lyme disease. Nymphs are very active from spring through early summer, at the height of outdoor activity for most people. Because they are still quite small (less than 2 mm), they are difficult to spot, giving them ample opportunity to transmit *B. burgdorferi* while feeding. Although far more adult ticks than nymphs carry *B. burgdorferi*, the adult ticks are much larger, more easily noticed, and more likely to be removed before the 24 hours or more of continuous feeding needed to transmit *B. burgdorferi*.

Lyme disease is a collection of effects caused by *B. burgdorferi*. Once the organism gains entry to the body through a tick bite, it can move through the bloodstream quickly. Only 12 hours after entering the bloodstream, *B. burgdorferi* can be found in cerebrospinal fluid (which means it can affect the nervous system). Treating Lyme disease early and thoroughly is important because *B. burgdorferi* can hide for long periods within the body in a clinically latent state. That ability explains why symptoms can recur in cycles and can flare up after months or years, even over decades. It is important to note, however, that not everyone exposed to *B. burgdorferi* develops the disease.

Lyme disease is usually described in terms of length of infection (time since the person was bitten by a tick infected with *B. burgdorferi*) and whether *B. burgdorferi* is localized or disseminated (spread through the body by fluids and cells carrying *B. burgdorferi*). Furthermore, when and how symptoms of Lyme disease appear can vary widely from patient to patient. People who experience recurrent bouts of symptoms over time are said to have chronic Lyme disease.

The most recognizable indicator of Lyme disease is a rash around the site of the tick bite. Often, the tick exposure has not been recognized. The eruption might be warm or itch. The rash, erythema migrans (EM), generally develops within 3–30 days and usually begins as a round, red patch that expands. Clearing may take place from the center out, leaving a bull's-eye effect; in some cases, the center gets redder instead of clearing. The rash may look like a bruise on people with dark skin. Of those who develop Lyme disease, about 50% notice the rash; about 50% notice flu-like symptoms, including fatigue, headache, chills and fever, muscle and joint pain, and lymph node swelling. However, a rash at the site can also be an allergic reaction to the tick saliva rather than an indicator of Lyme disease, particularly if the rash appears in less than three days and disappears only days later.

Weeks, months, or even years after an untreated tick bite, symptoms can appear in several forms, including fatigue, neurological problems, such as pain (unexplained and not triggered by an injury), Bell's palsy (facial paralysis, usually one-sided but may be on both sides), mimicking of the **inflammation** of brain membranes known as **meningitis** (fever, severe headache, stiff neck), and arthritis (short episodes of pain and swelling in joints). Less common effects of Lyme disease are heart abnormalities (such as irregular rhythm or cardiac block) and eye abnormalities (such as swelling of the cornea, tissue, or eye muscles and nerves).

A clear diagnosis of Lyme disease can be difficult, and relies on information the patient provides and the doctor's clinical judgment, particularly through elimination of other possible causes of the symptoms. Lyme disease may mimic other conditions, including chronic fatigue syndrome (CFS), multiple sclerosis (MS), and other diseases with many symptoms involving multiple body systems. Differential diagnosis (distinguishing Lyme disease from other diseases) is based on clinical evaluation with laboratory tests used for clarification, when necessary. A two-test approach is common to confirm the results. Because of the potential for misleading results (false-positive and false-negative), laboratory tests alone cannot establish the diagnosis.

Physicians generally know which disease-causing organisms are common in their geographic area. The most helpful piece of information is whether a tick bite or rash was noticed and whether it happened locally or while traveling. Doctors may not consider Lyme disease if it is rare locally, but will take it into account if a patient mentions vacationing in an area where the disease is commonly found.

The treatment for Lyme disease is antibiotic therapy. If a patient has strong indications of Lyme disease (symptoms and medical history), the doctor will probably begin treatment on the presumption of this disease. The American College of Physicians recommends treatment for a patient with a rash resembling EM or who has arthritis, a history of an EM-type rash, and a previous tick bite.

The physician may have to adjust the treatment regimen or change medications based on the patient's response. Treatment can be difficult because *B. burgdorferi* comes in several strains (some may react to different antibiotics than others) and may even have the ability to switch forms during the course of infection. Also, *B. burgdorferi* can shut itself up in cell niches, allowing it to elude antibiotic actions. Finally, antibiotics can kill *B. burgdorferi* only while it is active rather than dormant.

If aggressive antibiotic therapy is given early, and the patient cooperates fully and sticks to the medication schedule, recovery should be complete. Only a small percentage of Lyme disease patients fail to respond or relapse (have recurring episodes). Most long-term effects of the disease result when diagnosis and treatment is delayed or missed. Co-infection with other infectious organisms spread by ticks in the same areas as *B. burgdorferi* (babesiosis and ehrlichiosis, for instance) may be responsible for treatment failures or more severe symptoms. Lyme disease has been responsible for deaths, but that is rare.

An genetically engineered **vaccine** for Lyme disease was made available in the United States in 1999. **Immunity** requires three injections, the first two given a month apart; a third injection given a year later. Clinical trials conducted in 1997 from a large study of 10,000 adults in many locations showed strong promise of the vaccine's safety and efficacy. The **Centers for Disease Control** recommends the vaccine for those who live and work in Lyme disease endemic areas, and who have repeated and prolonged exposure to tick-infested areas (e.g., park rangers, landscape workers). The Lyme disease vaccine will not prevent other diseases spread by ticks, however, so protective measures against tick bites should still be observed. The vaccine is not recommended for travelers who will have little exposure when visiting areas where Lyme disease has occurred.

Precautions to avoid contact with ticks include moving leaves and brush away from living quarters. Most important are personal protection techniques when outdoors, such as using **repellents** containing DEET, wearing light-colored clothing to maximize ability to see ticks, tucking pant legs into socks or boot top, and checking children frequently for ticks.

LYMPHOCYTES • *see* T CELLS OR T LYMPHOCYTES

LYSOGENY

Lysogeny refers to a process whereby a virus that specifically infects a bacterium, a **bacteriophage** (which means "devourer of bacteria"), achieves the manufacture of copies of its **deoxyribonucleic acid** (**DNA**) genetic material by integrating the viral DNA into the DNA of the host **bacteria**. The inserted viral DNA is then replicated along with the host DNA.

The nature of lysogeny remained unresolved for many years following the discovery of the bacteriophage by Felix d'Hérelle in 1915. The sudden appearance of virus in cultures of bacteria was at first thought to be the result of viral **contamination**. The acceptance of lysogeny as a real phenomenon came almost 40 years later.

In lysogeny no new virus particles are made. Instead, the virus essentially remains dormant, while ensuring that its genetic material continues to be made. A stress to the bacterium, such as exposure of the bacterium to ultraviolet light, triggers the viral DNA to separate from the bacterial DNA. Then, new virus particles will form in what is known as the lytic cycle. The two processes of lysogeny and lysis are under a system of control first explained by the French biologist André Lwoff in the early 1950s.

Lysogeny is of benefit to the virus, allowing the genetic material to persist in the absence of a virus manufacture. Lysogeny can also be beneficial to the host bacterium. The primary benefit to bacteria occurs when the integrated viral DNA contains a **gene** that encodes a toxin. Possession of the toxin can be advantageous to those bacteria that establish an infection as part of their strategy of replication. For example, toxins encoded by bacteriophage genes are the main cause of the symptoms associated with the bacteria diseases of **tetanus**, **diphtheria**, and cholera.

The process of lysogeny has been studied most intensively in a bacteriophage that is designated as lambda. In the lambda bacteriophage, the establishment of lysogeny depends on the presence of three viral proteins. These are designated cI ("c-one"), cII, and cIII. The cI protein is manufactured first, using host molecules that interpret the information for the protein contained in the viral DNA, following the entry of the viral DNA into the host bacterium. At this point the viral DNA is not integrated into the host genome, but exists as an independent circle. CI is a so-called repressor protein that operates to occupy sequences on the viral genome that would otherwise be used to make the various viral proteins that are needed to assemble the new virus particles. By occupying these sites, cI prevents viral proteins from being produced.

At about the same time, the viral DNA becomes integrated into the host DNA and the cII and cIII proteins are manufactured. These latter proteins assist cI in the task of blocking synthesis of viral components. Accordingly, cI, cII, and cIII function to maintain the lysogenic state. The cII protein functions to make the manufacture of cI by the host's **transcription** machinery more efficient. The cIII protein helps protect the cII protein from being degraded by host **enzymes**.

Once lysogeny is established, the continued manufacture of the cI protein will maintain the integrated state of the viral DNA.

The cI protein maintains its own transcription. The binding of cI to a certain stretch of DNA promotes the recognition and use of the gene for cI to manufacture the cI protein. This is known as positive control. As well, the protein exerts a

negative control of another protein (termed "cro"). In negative control, the binding of cI to a region of the DNA prevents the gene from cro from being recognized and used to manufacture the cro protein.

The "decision" to maintain lysogeny or to begin the cycle whereby new virus particles are made and the bacterium explosively releases the new particles is essentially a competition between the cI and cro proteins. This competition centers on the binding of the proteins to a stretch of DNA called the O_R operator. This stretch of DNA has three sites that the proteins can occupy. Depending on which sites are occupied by which protein, the manufacture of either the cI or the cro proteins is promoted. If more cI is made, lysogeny continues. If cro is made, the process of viral assembly (i.e., the lytic cycle) begins. The lytic cycle can be triggered by events that damage the host bacterium, including exposure to environmental stressors (e.g., ultraviolet radiation exposure).

See also Bacteriophage and bacteriophage typing; Operon; Viral genetics; Virus replication

LYSOSOME

Lysosomes are small membranous bags of digestive **enzymes** found in the **cytoplasm** of all eukaryotic cells (those with true nuclei). As the principle site of intracellular digestion, they contain a variety of enzymes capable of degrading proteins, nucleic acids, sugars, lipids, and most other ordinary cellular components. These enzymes hydrolyze (break down) their target compounds best under acidic conditions. Although lysosomes vary considerably in size even within a single cell, the normal range is usually slightly smaller than the average mitochondrion.

The membrane enclosing lysosomes appears to be similar to that of other cellular organelles, but it has several unique properties. First, hydrogen pumps in the membrane acidify the lysosomal interior to a **pH** of five, an optimal level for the activity of its internal enzymes. The membrane has docking sites on its exterior that allow both materials to be digested and the enzymes to carry out the job to be transferred into the lysosome from transport vesicles derived from the Golgi apparatus, the endoplasmic reticulum, or from endocytosis by the plasma membrane. The lysosomal membrane also has transport complexes that allow the final products of digestion such as amino acids, simple sugars, salts, and nucleic acids to be exported back into the cytoplasm, where they can be either excreted or recycled by the cell into new cellular components. Finally, by mechanisms that are not yet fully understood, the lysosomal membrane is able to avoid digestion by the enzymes it contains even though it is composed of the same compounds that those enzymes routinely destroy.

See also Cell membrane transport

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MacLeod, Colin Munro (1909-1972)

Canadian-born American microbiologist

Colin Munro MacLeod is recognized as one of the founders of **molecular biology** for his research concerning the role of **deoxyribonucleic acid** (**DNA**) in **bacteria**. Along with his colleagues Oswald Avery and **Maclyn McCarty**, MacLeod conducted experiments on bacterial **transformation** which indicated that DNA was the active agent in the genetic transformation of bacterial cells. His earlier research focused on the causes of **pneumonia** and the development of serums to treat it. MacLeod later became chairman of the department of microbiology at New York University; he also worked with a number of government agencies and served as White House science advisor to President John F. Kennedy.

MacLeod, the fourth of eight children, was born in Port Hastings, in the Canadian province of Nova Scotia. He was the son of John Charles MacLeod, a Scottish Presbyterian minister, and Lillian Munro MacLeod, a schoolteacher. During his childhood, MacLeod moved with his family first to Saskatchewan and then to Quebec. A bright youth, he skipped several grades in elementary school and graduated from St. Francis College, a secondary school in Richmond, Quebec, at the age of fifteen. MacLeod was granted a scholarship to McGill University in Montreal but was required to wait a year for admission because of his age; during that time he taught elementary school. After two years of undergraduate work in McGill's premedical program, during which he became managing editor of the student newspaper and a member of the varsity ice hockey team, MacLeod entered the McGill University Medical School, receiving his medical degree in 1932.

Following a two-year internship at the Montreal General Hospital, MacLeod moved to New York City and became a research assistant at the Rockefeller Institute for Medical Research. His research there, under the direction of Oswald Avery, focused on pneumonia and the Pneumococcal infections which cause it. He examined the use of animal antiserums (liquid substances that contain proteins that guard against antigens) in the treatment of the disease. MacLeod also studied the use of **sulfa drugs**, synthetic substances that counteract bacteria, in treating pneumonia, as well as how Pneumococci develop a resistance to sulfa drugs. He also worked on a mysterious substance then known as "C-reactive protein," which appeared in the blood of patients with acute infections.

MacLeod's principal research interest at the Rockefeller Institute was the phenomenon known as bacterial transformation. First discovered by Frederick Griffith in 1928, this was a phenomenon in which live bacteria assumed some of the characteristics of dead bacteria. Avery had been fascinated with transformation for many years and believed that the phenomenon had broad implications for the science of biology. Thus, he and his associates, including MacLeod, conducted studies to determine how the bacterial transformation worked in Pneumococcal cells.

The researchers' primary problem was determining the exact nature of the substance which would bring about a transformation. Previously, the transformation had been achieved only sporadically in the laboratory, and scientists were not able to collect enough of the transforming substance to determine its exact chemical nature. MacLeod made two essential contributions to this project: He isolated a strain of *Pneumococcus* which could be consistently reproduced, and he developed an improved nutrient **culture** in which adequate quantities of the transforming substance could be collected for study.

By the time MacLeod left the Rockefeller Institute in 1941, he and Avery suspected that the vital substance in these transformations was DNA. A third scientist, Maclyn McCarty, confirmed their hypothesis. In 1944, MacLeod, Avery, and McCarty published "Studies of the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types: Induction of Transformation by a Deoxyribonucleic Acid Fraction Isolated from *Pneumococcus* Type III" in the *Journal of Experimental Medicine*. The article proposed that DNA was the material which brought about genetic transformation. Though the scientific community was slow to recognize the

article's significance, it was later hailed as the beginning of a revolution that led to the formation of molecular biology as a scientific discipline.

MacLeod married Elizabeth Randol in 1938; they eventually had one daughter. In 1941, MacLeod became a citizen of the United States, and was appointed professor and chairman of the department of microbiology at the New York University School of Medicine, a position he held until 1956. At New York University he was instrumental in creating a combined program in which research-oriented students could acquire both an M.D. and a Ph.D. In 1956, he became professor of research medicine at the Medical School of the University of Pennsylvania. MacLeod returned to New York University in 1960 as professor of medicine and remained in that position until 1966.

From the time the United States entered World War II until the end of his life, MacLeod was a scientific advisor to the federal government. In 1941, he became director of the Commission on Pneumonia of the United States Army Epidemiological Board. Following the unification of the military services in 1949, he became president of the Armed Forces Epidemiological Board and served in that post until 1955. In the late 1950s, MacLeod helped establish the Health Research Council for the City of New York and served as its chairman from 1960 to 1970. In 1963, President John F. Kennedy appointed him deputy director of the Office of Science and Technology in the Executive Office of the President; from this position he was responsible for many program and policy initiatives, most notably the United States/Japan Cooperative Program in the Medical Sciences.

In 1966, MacLeod became vice-president for Medical Affairs of the Commonwealth Fund, a philanthropic organization. He was honored by election to the National Academy of Sciences, the American Philosophical Society, and the American Academy of Arts and Sciences. MacLeod was en route from the United States to Dacca, Bangladesh, to visit a cholera laboratory when he died in his sleep in a hotel at the London airport in 1972. In the *Yearbook of the American Philosophical Society,* Maclyn McCarty wrote of MacLeod's influence on younger scientists, "His insistence on rigorous principles in scientific research was not enforced by stern discipline but was conveyed with such good nature and patience that it was simply part of the spirit of investigation in his laboratory."

See also Bacteria and bacterial infection; Microbial genetics; Pneumonia, bacterial and viral

MAD COW DISEASE • *see* BSE AND CJD DISEASE

MAGNETOTACTIC BACTERIA

Magnetotactic **bacteria** are bacteria that use the magnetic field of Earth to orient themselves. This phenomenon is known as magnetotaxis. Magnetotaxis is another means by which bacteria can actively respond to their environment. Response to light (phototaxis) and chemical concentration (chemotaxis) exist in other species of bacteria.

The first magnetotactic bacterium, *Aquasprilla magnetotactum* was discovered in 1975 by Richard Blakemore. This organism, which is now called *Magnetospirillum magnetotacticum*, inhabits swampy water, where because of the decomposition of organic matter, the oxygen content in the water drops off sharply with increasing depth. The bacteria were shown to use the magnetic field to align themselves. By this behavior, they were able to position themselves at the region in the water where oxygen was almost depleted, the environment in which they grow best. For example, if the bacteria stray too far above or below the preferred zone of habitation, they reverse their direction and swim back down or up the lines of the magnetic field until they reach the preferred oxygen concentration. The bacteria have flagella, which enables them to actively move around in the water. Thus, the sensory system used to detect oxygen concentration is coordinated with the movement of the flagella.

Magnetic orientation is possible because the magnetic North Pole points downward in the Northern Hemisphere. So, magnetotactic bacteria that are aligned to the fields are also pointing down. In the Northern Hemisphere, the bacteria would move into oxygen-depleted water by moving north along the field. In the Southern Hemisphere, the magnetic North Pole points up and at an angle. So, in the Southern Hemisphere, magnetotactic bacteria are south-seeking and also point downward. At the equator, where the magnetic North Pole is not oriented up or down, magnetotactic bacteria from both hemispheres can be found.

Since the initial discovery in 1975, magnetotactic bacteria have been found in freshwater and salt water, and in oxygen rich as well oxygen poor zones at depths ranging from the near-surface to 2000 meters beneath the surface. Magnetotactic bacteria can be spiral-shaped, rods and spheres. In general, the majority of magnetotactic bacteria discovered so far gather at the so-called oxic-anoxic transition zone; the zone above which the oxygen content is high and below which the oxygen content is essentially zero.

Magnetotaxis is possible because the bacteria contain magnetically responsive particles inside. These particles are composed of an iron-rich compound called magnetite, or various iron and sulfur containing compounds (ferrimagnetite greigite, pyrrhotite, and pyrite). Typically, these compounds are present as small spheres arranged in a single chain or several chains (the maximum found so far is five) in the **cytoplasm** of each bacterium. The spheres are enclosed in a membrane. This structure is known as a magnetosome. Since many bacterial membranes selectively allow the movement of molecules across them, magnetosome membranes may function to create a unique environment within the bacterial cytoplasm in which the magnetosome crystal can form. The membranes may also be a means of extending the chain of magnetosome, with a new magnetosome forming at the end of the chain.

Magnetotactic bacteria may not inhabit just Earth. Examination of a 4.5 billion-year-old Martian meteorite in

2000 revealed the presence of magnetite crystals, which on Earth are produced only in magnetotactic bacteria. The magnetite crystals found in the meteorite are identical in shape, size and composition to those produced in *Magnetospirillum magnetotacticum*. Thus, magnetite is a "biomarker," indicating that life may have existed on Mars in the form of magnetotactic bacteria. The rationale for the use of magnetotaxis in Martian bacteria is still a point of controversy. The Martian atmosphere is essentially oxygen-free and the magnetic field is nearly one thousand times weaker than on Earth.

Magnetotactic bacteria are also of scientific and industrial interest because of the quality of their magnets. Bacterial magnets are much better in performance than magnets of comparable size that are produced by humans. Substitution of man-made micro-magnets with those from magnetotactic bacteria could be both feasible and useful.

See also Bacterial movement

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

In humans, the proteins coded by the genes of the major **histocompatibility** complex (MHC) include human leukocyte antigens (**HLA**), as well as other proteins. HLA proteins are present on the surface of most of the body's cells and are important in helping the **immune system** distinguish "self" from "non-self" molecules, cells, and other objects.

The function and importance of MHC is best understood in the context of a basic understanding of the function of the immune system. The immune system is responsible for distinguishing foreign proteins and other antigens, primarily with the goal of eliminating foreign organisms and other invaders that can result in disease. There are several levels of defense characterized by the various stages and types of immune response.

Present on chromosome 6, the major histocompatibility complex consists of more than 70 genes, classified into class I, II, and III MHC. There are multiple alleles, or forms, of each HLA **gene**. These alleles are expressed as proteins on the surface of various cells in a co-dominant manner. This diversity is important in maintaining an effective system of specific **immunity**. Altogether, the MHC genes span a region that is four million base pairs in length. Although this is a large region, 99% of the time these closely linked genes are transmitted to the next generation as a unit of MHC alleles on each chromosome 6. This unit is called a haplotype.

Class I MHC genes include HLA-A, HLA-B, and HLA-C. Class I MHC are expressed on the surface of almost all cells. They are important for displaying **antigen** from **viruses** or **parasites** to killer T-cells in cellular immunity. Class I MHC is also particularly important in organ and tissue rejection following transplantation. In addition to the portion of class I MHC coded by the genes on chromosome 6, each class I MHC protein also contains a small, non-variable protein component called beta 2-microglobulin coded by a gene on chromosome 15. Class I HLA genes are highly polymorphic, meaning there are multiple forms, or alleles, of each gene. There are at least 57 HLA-A alleles, 111 HLA-B alleles, and 34 HLA-C alleles.

Class II MHC genes include HLA-DP, HLA-DQ, and HLA-DR. Class II MHC are particularly important in humoral immunity. They present foreign antigen to helper T-cells, which stimulate B-cells to elicit an **antibody** response. Class II MHC is only present on antigen presenting cells, including phagocytes and B-cells. Like Class I MHC, there are hundreds of alleles that make up the class II HLA gene pool.

Class III MHC genes include the **complement** system (i.e. C2, C4a, C4b, Bf). Complement proteins help to activate and maintain the inflammatory process of an immune response.

When a foreign organism enters the body, it is encountered by the components of the body's natural immunity. Natural immunity is the non-specific first-line of defense carried out by phagocytes, natural killer cells, and components of the complement system. Phagocytes are specialized white blood cells that are capable of engulfing and killing an organism. Natural killer cells are also specialized white blood cells that respond to cancer cells and certain viral infections. The complement system is a group of proteins called the class III MHC that attack antigens. Antigens consist of any molecule capable of triggering an immune response. Although this list is not exhaustive, antigens can be derived from toxins, protein, carbohydrates, **DNA**, or other molecules from viruses, **bacteria**, cellular parasites, or cancer cells.

The natural immune response will hold an infection at bay as the next line of defense mobilizes through acquired, or specific, immunity. This specialized type of immunity is usually what is needed to eliminate an infection and is dependent on the role of the proteins of the major histocompatibility complex. There are two types of acquired immunity. Humoral immunity is important in fighting infections outside the body's cells, such as those caused by bacteria and certain viruses. Other **types of viruses** and parasites that invade the cells are better fought by cellular immunity. The major players in acquired immunity are the antigen-presenting cells (APCs), B-cells, their secreted antibodies, and the T-cells. Their functions are described in detail below.

In humoral immunity, antigen-presenting cells, including some B-cells, engulf and break down foreign organisms. Antigens from these foreign organisms are then brought to the outside surface of the antigen-presenting cells and presented in conjunction with class II MHC proteins. The helper T-cells recognize the antigen presented in this way and release **cytokines**, proteins that signal B-cells to take further action. B-cells are specialized white blood cells that mature in the bone marrow. Through the process of maturation, each B-cell develops the ability to recognize and respond to a specific antigen. Helper T-cells aid in stimulating the few B-cells that can recognize a particular foreign antigen. B-cells that are stimulated in this way develop into plasma cells, which secrete antibodies specific to the recognized antigen. Antibodies are proteins that are present in the circulation, as well as being bound to the surface of B-cells. They can destroy the foreign organism from which the antigen came. Destruction occurs either directly, or by tagging the organism, which will then be more easily rec-

ognized and targeted by phagocytes and complement proteins. Some of the stimulated B-cells go on to become memory cells, which are able to mount an even faster response if the antigen is encountered a second time.

Another type of acquired immunity involves killer T-cells and is termed cellular immunity. T-cells go through a process of maturation in the organ called the thymus, in which T-cells that recognized self-antigens are eliminated. Each remaining T-cell has the ability to recognize a single, specific, non-self antigen that the body may encounter. Although the names are similar, killer T-cells are unlike the non-specific natural killer cells in that they are specific in their action. Some viruses and parasites quickly invade the body's cells, where they are hidden from antibodies. Small pieces of proteins from these invading viruses or parasites are presented on the surface of infected cells in conjunction with class I MHC proteins, which are present on the surface of most all of the body's cells. Killer T-cells can recognize antigen bound to class I MHC in this way, and they are prompted to release chemicals that act directly to kill the infected cell. There is also a role for helper T-cells and antigen-presenting cells in cellular immunity. Helper T-cells release cytokines, as in the humoral response, and the cytokines stimulate killer T-cells to multiply. Antigen-presenting cells carry foreign antigen to places in the body where additional killer T-cells can be alerted and recruited.

The major histocompatibility complex clearly performs an important role in functioning of the immune system. Related to this role in disease immunity, MHC is also important in organ and tissue transplantation, as well as playing a role in susceptibility to certain diseases. HLA typing can also provide important information in parentage, forensic, and anthropologic studies.

There is significant variability of the frequencies of HLA alleles among ethnic groups. This is reflected in anthropologic studies attempting to use HLA-types to determine patterns of migration and evolutionary relationships of peoples of various ethnicity. Ethnic variation is also reflected in studies of HLA-associated diseases. Generally, populations that have been subject to significant patterns of migration and assimilation with other populations tend to have a more diverse HLA gene pool. For example, it is unlikely that two unrelated individuals of African ancestry would have matched HLA types. Conversely, populations that have been isolated due to geography, cultural practices, and other historical influences may display a less diverse pool of HLA types, making it more likely for two unrelated individuals to be HLA-matched.

There is a role for HLA typing of individuals in various settings. Most commonly, HLA typing is used to establish if an organ or tissue donor is appropriately matched to the recipient for key HLA types, so as not to elicit a rejection reaction in which the recipient's immune system attacks the donor tissue. In the special case of bone marrow transplantation, the risk is for graft-versus-host disease (GVHD), as opposed to tissue rejection. Because the bone marrow contains the cells of the immune system, the recipient effectively receives the donor's immune system. If the donor immune system recognizes the recipient's tissues as foreign, it may begin to attack, causing the inflammatory and other complications of GVHD. As advances occur in transplantation medicine, HLA typing for transplantation occurs with increasing frequency and in various settings.

There is an established relationship between the inheritance of certain HLA types and susceptibility to specific diseases. Most commonly, these are diseases that are thought to be autoimmune in nature. Autoimmune diseases are those characterized by inflammatory reactions that occur as a result of the immune system mistakenly attacking self tissues. The basis of the HLA association is not well understood, although there are some hypotheses. Most autoimmune diseases are characterized by the expression of class II MHC on cells of the body that do not normally express these proteins. This may confuse the killer T-cells, which respond inappropriately by attacking these cells. Molecular mimicry is another hypothesis. Certain HLA types may look like antigens from foreign organisms. If an individual is infected by such a foreign virus or bacteria, the immune system mounts a response against the invader. However, there may be a cross-reaction with cells displaying the HLA type that is mistaken for foreign antigen. Whatever the underlying mechanism, certain HLA-types are known factors that increase the relative risk for developing specific autoimmune diseases. For example, individuals who carry the HLA B-27 allele have a relative risk of 150 for developing ankylosing spondylitis—meaning such an individual has a 150-fold chance of developing this form of spinal and pelvic arthritis, as compared to someone in the general population. Selected associations are listed below (disease name is first, followed by MHC allele and then the approximate corresponding relative risk of disease).

- Type 1 diabetes, DR3, 5
- Type 1 diabetes, DR4, 5
- Type 1 diabetes, DR3 + DR4, 20-40
- Narcolepsy, DR2, 260-360
- Ankylosing spondylitis, B27, 80-150
- Reiter's disease, B27, 37
- Rheumatoid arthritis, DR4, 3-6
- Myasthenia gravis, B8, 4
- Lupus, DR3, 2
- Graves disease, DR3, 5
- Multiple sclerosis, DR2, 3
- Celiac disease, DR3 and DR7, 5-10
- Psoriasis vulgaris, Cw6, 8

In addition to autoimmune disease, HLA-type less commonly plays a role in susceptibility to other diseases, including cancer, certain infectious diseases, and metabolic diseases. Conversely, some HLA-types confer a protective advantage for certain types of infectious disease. In addition, there are rare immune deficiency diseases that result from inherited **mutations** of the genes of components of the major histocompatibility complex.

Among other tests, HLA typing can sometimes be used to determine parentage, most commonly paternity, of a child. This type of testing is not generally done for medical reasons, but rather for social or legal reasons.

HLA-typing can provide valuable DNA-based evidence contributing to the determination of identity in criminal cases. This technology has been used in domestic criminal trials. Additionally, it is a technology that has been applied internationally in the human-rights arena. For example, HLA-typing had an application in Argentina following a military dictatorship that ended in 1983. The period under the dictatorship was marked by the murder and disappearance of thousands who were known or suspected of opposing the regime's practices. Children of the disappeared were often adopted by military officials and others. HLA-typing was one tool used to determine non-parentage and return children of the disappeared to their biological families.

HLA-typing has proved to be an invaluable tool in the study of the evolutionary origins of human populations. This information, in turn, contributes to an understanding of cultural and linguistic relationships and practices among and within various ethnic groups.

See also Antibody and antigen; Immunity, cell mediated; Immunity, humoral regulation; Immunodeficiency disease syndromes; Immunodeficiency diseases; Immunogenetics; Immunological analysis techniques; Transplantation genetics and immunology

MALARIA AND THE PHYSIOLOGY OF PARASITIC INFECTIONS

Malaria is a disease caused by a unicellular parasite known as *Plasmodium.* Although more than 100 different species of *Plasmodium* exist, only four types are known to infect humans including, *Plasmodium falciparum, vivax, malariae,* and *ovale*. While each type has a distinct appearance under the **microscope**, they each can cause a different pattern of symptoms. *Plasmodium falciparum* is the major cause of death in Africa, while *Plasmodium vivax* is the most geographically widespread of the species and the cause of most malaria cases diagnosed in the United States. *Plasmodium malariae* infections produce typical malaria symptoms that persist in the blood for very long periods, sometimes without ever producing symptoms. *Plasmodium ovale* is rare, and is isolated to West Africa. Obtaining the complete sequence of the *Plasmodium* genome is currently under way.

The life cycle of *Plasmodium* relies on the insect host (for example, the Anopheles mosquito) and the carrier host (humans) for its propagation. In the insect host, the *Plasmodium* parasite undergoes sexual reproduction by uniting two sex cells producing what are called sporozoites. When an infected mosquito feeds on human blood, the sporozoites enter into the bloodstream. During a mosquito bite, the saliva containing the infectious sporozoite from the insect is injected into the bloodstream of the human host and the blood that the insect removes provides nourishment for her eggs. The parasite immediately is targeted for a human liver cell, where it can escape from being destroyed by the **immune system**. Unlike in the insect host, when the sporozoite infects a single liver cell from the human host, it can undergo asexual reproduction (multiple rounds consisting of replication of the **nucleus** followed by budding to form copies of itself).

During the next 72 hours, a sporozoite develops into a schizont, a structure containing thousands of tiny rounded merozoites. Schizont comes from the Greek word *schizo,* meaning to tear apart. One infectious sporozoite can develop into 20,000 merozoites. Once the schizont matures, it ruptures the liver cells and leaks the merozoites into the bloodstream where they attack neighboring erythrocytes (red blood cells, RBC). It is in this stage of the parasite life cycle that disease and death can be caused if not treated. Once inside the **cytoplasm** of an erythrocyte, the parasite can break down hemoglobin (the primary oxygen transporter in the body) into amino acids (the building blocks that makeup protein). A by-product of the degraded hemoglobin is hemozoin, or a pigment produced by the breakdown of hemoglobin. Golden-brown to black granules are produced from hemozoin and are considered to be a distinctive feature of a blood-stage parasitic infection. The blood-stage **parasites** produce schizonts, which rupture the infected erythrocytes, releasing many waste products, explaining the intermittent fever attacks that are associated with malaria.

The propagation of the parasite is ensured by a certain type of merozoite, that invades erythrocytes but does not asexually reproduce into schizonts. Instead, they develop into gametocytes (two different forms or sex cells that require the union of each other in order to reproduce itself). These gametocytes circulate in the human's blood stream and remain quiescent (dormant) until another mosquito bite, where the gametocytes are fertilized in the mosquito's stomach to become sporozoites. Gametocytes are not responsible for causing disease in the human host and will disappear from the circulation if not taken up by a mosquito. Likewise, the salivary sporozoites are not capable of re-infecting the salivary gland of another mosquito. The cycle is renewed upon the next feeding of human blood. In some types of *Plasmodium,* the sporozoites turn into hypnozoites, a stage in the life cycle that allows the parasite to survive but in a dormant phase. A relapse occurs when the hypnozoites are reverted back into sporozoites.

An infected erythrocyte has knobs on the surface of the cells that are formed by proteins that the parasite is producing during the schizont stage. These knobs are only found in the schizont stage of *Plasmodium falciparum* and are thought to be contacted points between the infected RBC and the lining of the blood vessels. The parasite also modifies the erythrocyte membrane itself with these knob-like structures protruding at the cell surface. These parasitic-derived proteins that provide contact points thereby avoid clearance from the blood stream by the spleen. Sequestration of schizont-infected erythrocytes to blood vessels that line vital organ such as the brain, lung, heart, and gut can cause many health-related problems.

A malaria-infected erythrocyte results in physiological alterations that involve the function and structure of the erythrocyte membrane. Novel parasite-induced permeation pathways (NPP) are produced along with an increase, in some cases, in the activity of specific transporters within the RBC. The NPP are thought to have evolved to provide the parasite

with the appropriate nutrients, explaining the increased permeability of many solutes. However, the true nature of the NPP remains an enigma. Possible causes for the NPP include 1) the parasite activates native transporters, 2) proteins produced by the parasite cause structural defects, 3) **plasmodium** inserts itself into the channel thus affecting it's function, and 4) the parasite makes the membrane more 'leaky'. The properties of the transporters and channels on a normal RBC differ dramatically from that of a malaria-infected RBC. Additionally, the lipid composition in terms of its fatty acid pattern is significantly altered, possibly due to the nature in which the parasite interacts with the membrane of the RBC. The dynamics of the membranes, including how the fats that makeup the membrane are deposited, are also altered. The increase in transport of solutes is bidirectional and is a function of the developmental stage of the parasite. In other words, the alterations in erythrocyte membrane are proportional to the maturation of the parasite.

See also Parasites

MARGULIS, LYNN (1938-)

American biologist

Lynn Margulis is a theoretical biologist and professor of botany at the University of Massachusetts at Amherst. Her research on the evolutionary links between cells containing nuclei (**eukaryotes**) and cells without nuclei (prokaryotes) led her to formulate a symbiotic theory of **evolution** that was initially spurned in the scientific community but has become more widely accepted.

Margulis, the eldest of four daughters, was born in Chicago. Her father, Morris Alexander, was a lawyer who owned a company that developed and marketed a long-lasting thermoplastic material used to mark streets and highways. He also served as an assistant state's attorney for the state of Illinois. Her mother, Leone, operated a travel agency. When Margulis was fifteen, she completed her second year at Hyde Park High School and was accepted into an early entrant program at the University of Chicago.

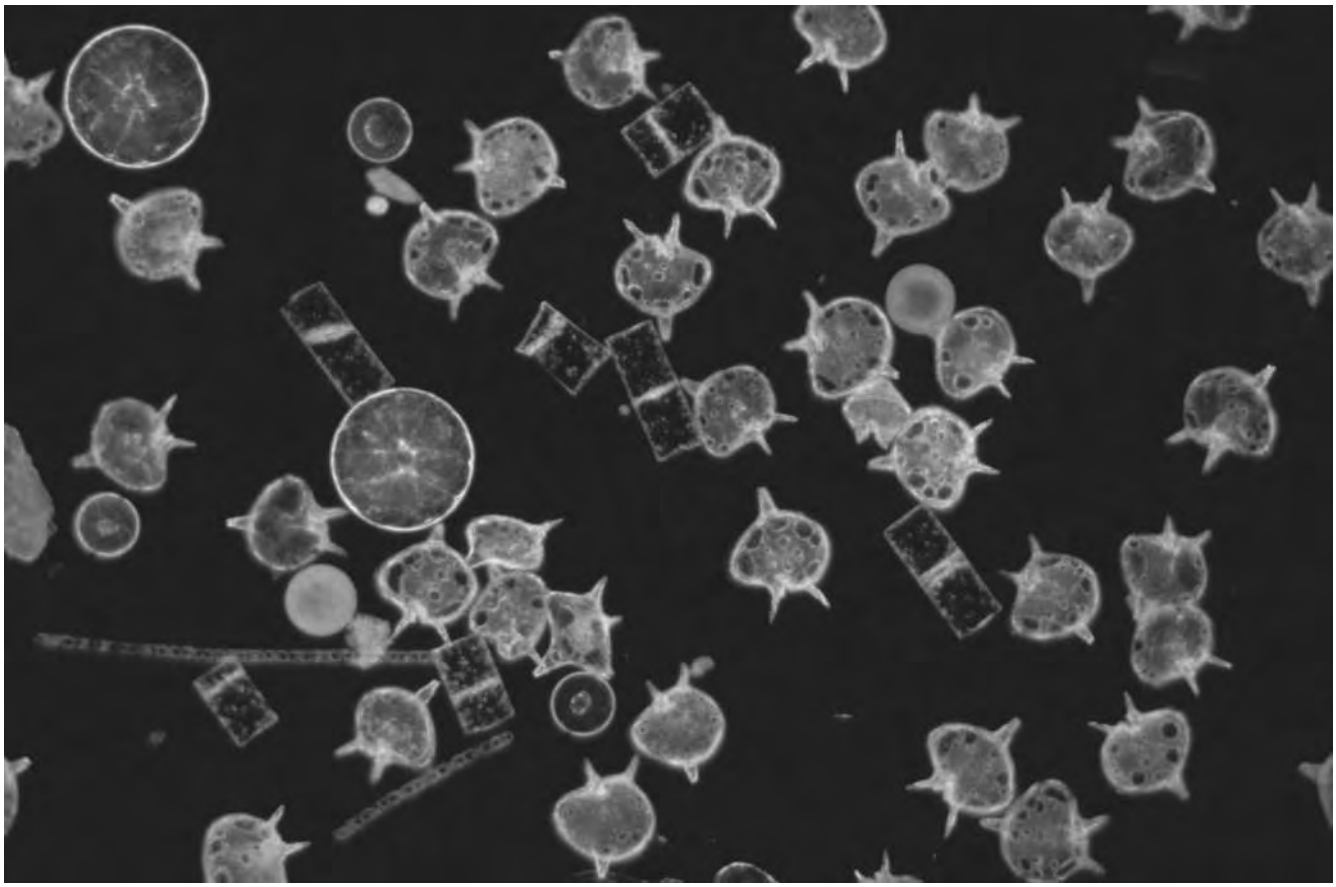
Margulis was particularly inspired by her science courses, in large part because reading assignments consisted not of textbooks but of the original works of the world's great scientists. A course in natural science made an immediate impression and would influence her life, raising questions that she has pursued throughout her career: What is heredity? How do genetic components influence the development of offspring? What are the common bonds between generations? While at the University of Chicago she met Carl Sagan, then a graduate student in physics. At the age of nineteen, she married Sagan, received a B.A. in liberal arts, and moved to Madison, Wisconsin, to pursue a joint master's degree in zoology and genetics at the University of Wisconsin under the guidance of noted cell biologist Hans Ris. In 1960, Margulis and Sagan moved to the University of California at Berkeley, where she conducted genetic research for her doctoral dissertation.

The marriage to Sagan ended before she received her doctorate. She moved to Waltham, Massachusetts, with her two sons, Dorion and Jeremy, to accept a position as lecturer in the department of biology at Brandeis University. She was awarded her Ph.D. in 1965. The following year, Margulis became an adjunct assistant of biology at Boston University, leaving 22 years later as full professor. In 1967, Margulis married crystallographer Thomas N. Margulis. The couple had two children before they divorced in 1980. Since 1988, Margulis has been a distinguished university professor with the Department of Botany at the University of Massachusetts at Amherst.

Margulis' interest in genetics and the development of cells can be traced to her earliest days as a University of Chicago undergraduate. She always questioned the commonly accepted theories of genetics, but also challenged the traditionalists by presenting hypotheses that contradicted current beliefs. Margulis has been called the most gifted theoretical biologist of her generation by numerous colleagues. A profile of Margulis by Jeanne McDermott in the *Smithsonian* quotes Peter Raven, director of the Missouri Botanical Garden and a MacArthur fellow: "Her mind keeps shooting off sparks. Some critics say she's off in left field. To me she's one of the most exciting, original thinkers in the whole field of biology." Although few know more about cellular biology, Margulis considers herself a "microbial evolutionist," mapping out a field of study that doesn't in fact exist.

As a graduate student, Margulis became interested in cases of non-Mendelian inheritance, occurring when the genetic make-up of a cell's descendants cannot be traced solely to the genes in a cell's **nucleus**. For several years, she concentrated her research on a search for genes in the **cytoplasm** of cells, the area outside of the cell's nucleus. In the early 1960s, Margulis presented evidence for the existence of extranuclear genes. She and other researchers had found **DNA** in the cytoplasm of plant cells, indicating that heredity in higher organisms is not solely determined by genetic information carried in the cell nucleus. Her continued work in this field led her to formulate the serial endosymbiotic theory, or SET, which offered a new approach to evolution as well as an account of the origin of cells with nuclei.

Prokaryotes—bacteria and **blue-green algae** now commonly referred to as cyanobacteria—are single-celled organisms that carry genetic material in the cytoplasm. Margulis proposes that eukaryotes (cells with nuclei) evolved when different kinds of prokaryotes formed symbiotic systems to enhance their chances for survival. The first such symbiotic fusion would have taken place between fermenting **bacteria** and oxygen-using bacteria. All cells with nuclei, Margulis contends, are derived from bacteria that formed symbiotic relationships with other primordial bacteria some two billion years ago. It has now become widely accepted that mitochondria—those components of eukaryotic cells that process oxygen—are remnants of oxygen-using bacteria. Margulis' hypothesis that cell hairs, found in a vast array of eukaryotic cells, descend from another group of primordial bacteria much like the modern spirochaete still encounters resistance, however.



Light microscopic view of marine plankton.

The resistance to Margulis' work in microbiology may perhaps be explained by its implications for the more theoretical aspects of evolutionary theory. Evolutionary theorists, particularly in the English-speaking countries, have always put a particular emphasis on the notion that competition for scarce resources leads to the survival of the most well-adapted representatives of a species by natural **selection**, favoring adaptive genetic **mutations**. According to Margulis, natural selection as traditionally defined cannot account for the "creative novelty" to be found in evolutionary history. She argues instead that the primary mechanism driving biological change is symbiosis, while competition plays a secondary role.

Margulis doesn't limit her concept of symbiosis to the origin of plant and animal cells. She subscribes to the Gaia hypothesis first formulated by James E. Lovelock, British inventor and chemist. The Gaia theory (named for the Greek goddess of Earth) essentially states that all life, as well as the oceans, the atmosphere, and Earth itself are parts of a single, all-encompassing symbiosis and may fruitfully be considered as elements of a single organism.

Margulis has authored more than one hundred and thirty scientific articles and ten books, several of which are written with her son Dorion. She has also served on more than two dozen committees, including the American Association for the Advancement of Science, the MacArthur Foundation Fellowship Nominating Committee, and the editorial boards of several scientific journals. Margulis is co-director of NASA's Planetary Biology Internship Program and, in 1983, was elected to the National Academy of Sciences.

See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Microbial genetics; Microbial symbiosis

MARINE MICROBIOLOGY

Marine microbiology refers to the study of the **microorganisms** that inhabit saltwater. Until the past two to three decades, the oceans were regarded as being almost devoid of microorganisms. Now, the importance of microorganisms such as **bacteria** to the ocean ecosystem and to life on Earth is increasingly being recognized.

Microorganisms such as bacteria that live in the ocean inhabit a harsh environment. Ocean temperatures are generally very cold—approximately 37.4° F (about 3° C) on average—and this temperature tends to remain the cold except in shallow areas. About 75% of the oceans of the world are below

3300 feet (1000 meters) in depth. The pressure on objects like bacteria at increasing depths is enormous.

Some marine bacteria have adapted to the pressure of the ocean depths and require the presence of the extreme pressure in order to function. Such bacteria are barophilic if their requirement for pressure is absolute or barotrophic if they can tolerate both extreme and near-atmospheric pressures. Similarly, many marine bacteria have adapted to the cold growth temperatures. Those which tolerate the temperatures are described as psychrotrophic, while those bacteria that require the cold temperatures are psychrophilic ("cold loving").

Marine waters are elevated in certain ions such as sodium. Not surprisingly, marine microbes like bacteria have an absolute requirement for sodium, as well as for potassium and magnesium ions. The bacteria have also adapted to grow on very low concentrations of nutrients. In the ocean, most of the organic material is located within 300 meters of the surface. Very small amounts of usable nutrients reach the deep ocean. The bacteria that inhabit these depths are in fact inhibited by high concentrations of organic material.

The bacterial communication system known as **quorum sensing** was first discovered in the marine bacterium *Vibrio fischeri*. An inhibitor of the quorum sensing mechanism has also been uncovered in a type of marine algae.

Marine microbiology has become the subject of much commercial interest. Compounds with commercial potential as nutritional additives and antimicrobials are being discovered from marine bacteria, actinomycetes and **fungi**. For example the burgeoning marine nutraceuticals market represents millions of dollars annually, and the industry is still in its infancy. As relatively little is still known of the marine microbial world, as compared to terrestrial microbiology, many more commercial and medically relevant compounds undoubtedly remain to be discovered.

See also Bacterial kingdoms; Bacterial movement; Biodegradable substances; Biogeochemical cycles

MARSHALL, BARRY J. (1951-)

Australian physician

Barry Marshall was born in Perth, Australia. He is a physician with a clinical and research interest in gastroenterology. He is internationally recognized for his discovery that the bacterium *Helicobacter pylori* is the major cause of stomach ulcers.

Marshall studied medicine at the University of Western Australia from 1969 to 1974. While studying for his medical degree, Marshall decided to pursue medical research. He undertook research in the laboratory of Dr. Robin Warren, who had observations of a helical **bacteria** in the stomach of people suffering from ulcers.

Marshall and Warren succeeded in culturing the bacterium, which they named *Helicobacter pylori*. Despite their evidence that the organism was the cause of stomach ulceration, the medical community of the time was not convinced that a bacterium could survive the harsh acidic conditions of the stomach yet alone cause tissue damage in this environment. In order to illustrate the relevance of the bacterium to the disease, Marshall performed an experiment that has earned him international renown. In July of 1984, he swallowed a solution of the bacterium, developed the infection, including **inflammation** of the stomach, and cured himself of both the infection and the stomach inflammation by antibiotic therapy.

By 1994, Marshall's theory of Helicobacter involvement in stomach ulcers was accepted, when the United States National Institutes of Health endorsed **antibiotics** s the standard treatment for stomach ulcers.

Since Marshall's discovery, *Helicobacter pylori* has been shown to be the leading cause of stomach and intestinal ulcers, gastritis and stomach cancer. Many thousands of ulcer patients around the world have been successfully treated by strategies designed to attack **bacterial infection**. Marshall's finding was one of the first indications that human disease thought to be due to biochemical or genetic defects were in fact due to bacterial infections.

From Australia, Marshall spent a decade at the University of Virginia, where he founded and directed the Center for Study of Diseases due to *H. pylori*. While at Virginia, he developed an enzyme-based rapid test for the presence of the bacterium that tests patient's breath. The test is commercially available.

Currently, he is a clinician and researcher at the Sir Charles Gairdner Hospital in Perth, Australia.

Marshall's discovery has been recognized internationally. He has received the Warren Alpert Prize from the Harvard Medical School, which recognizes work that has most benefited clinical practice. Also, he has won the **Paul Ehrlich** Prize (Germany) and the Lasker Prize (United States).

See also Bacteria and bacterial infection; Helicobacteriosis

MASTIGOPHORA

Mastigophora is a division of single-celled protozoans. There are approximately 1,500 species of Mastigophora. Their habitat includes fresh and marine waters. Most of these species are capable of self-propelled movement through the motion of one or several flagella. The possession of flagella is a hallmark of the Mastigophora.

In addition to their flagella, some mastigophora are able to extend their interior contents (that is known as **cytoplasm**) outward in an arm-like protrusion. These protrusions, which are called pseudopodia, are temporary structures that serve to entrap and direct food into the microorganism. The cytoplasmic extensions are flexible and capable of collapsing back to form the bulk of the wall that bounds the microorganism.

Mastigophora replicate typically by the internal duplication of their contents flowed by a splitting of the microbes to form two daughter cells. This process, which is called binary fission, is analogous to the division process in **bacteria**. In addition to replicating by binary fission, some mastigophora can reproduce sexually, by the combining of genetic material from two mastigophora. This process is referred to as syngamy.

The mastigophora are noteworthy mainly because of the presence in the division of several disease-causing species. Some mastigophora are **parasites**, which depend on the infection of a host for the completion of their life cycle. These parasites cause disease in humans and other animals. One example is the Trypanosomes, which cause African **sleeping sickness** and Chaga's disease. Another example is *Giardia lamblia*. This microorganism is the agent that causes an intestinal malady called giardiasis. The condition has also been popularly dubbed "beaver fever," reflecting its presence in the natural habitat, where it is a resident of the intestinal tract of warm-blooded animals.

Giardia lamblia is an important contaminant of drinking water. The microorganism is resistant to the disinfectant action of chlorine, which is the most common chemical for the treatment of drinking water. In addition, a dormant form of the microorganism called a cyst is small enough that it can elude the filtration step in water treatment plants. The microbe is increasingly becoming a concern in drinking waters all over the world, even in industrialized countries with state of the art water treatment infrastructure.

See also Protozoa

MATIN, A. C. (1941-)

Indian American microbiologist

A. C. Matin is a Professor of Microbiology and **Immunology** at Stanford University in Stanford, California. He has made pioneering contributions to microbiology in a number of areas; these include his notable research into the ways in which **bacteria** like ***Escherichia coli*** adapt and survive periods of nutrient starvation. His studies have been important in combating infections and the remediation of wastes.

Matin was born in Delhi, India. He attended the University of Karachi, where he received his B.S. in microbiology and zoology in 1960 and his M.S. in microbiology in 1962. From 1962 until 1964 he was a lecturer in microbiology at St. Joseph's College for Women in Karachi. He then moved to the United States to attend the University of California at Los Angeles, from which he received a Ph.D. in microbiology (with distinction) in 1969. From 1969 until 1971 he was a postdoctoral research associate at the State University of The Netherlands. He then became a Scientific Officer, First Class, in the Department of Microbiology at the same institution, a post he held until 1975. That year Matin returned to the United States to accept a position at Stanford University, the institution with which he remains affiliated.

Matin has made fundamental contributions to the biochemical and molecular biological study of the bacterial stress response—that is, how bacteria adapt to stresses in parameters such as temperature, **pH** (a measure of the acidity and alkalinity of a solution), and food availability. Matin and his colleagues provided much of the early data on the behavior of bacteria when their nutrients begin to become exhausted and waste products accumulate. This phase of growth, termed the stationary phase, has since been shown to have great relevance to the growth conditions that disease-causing bacteria face in the body, and which bacteria can face in the natural environment.

Matin has also made important contributions to the study of multidrug resistance in the bacterium *Escherichia coli*, specifically the use of a protein pump to exclude a variety of antibacterial drugs, and to the **antibiotic resistance** of *Staphylococcus aureus*.

Matin has published over 70 major papers and over 30 book chapters and articles. He has consulted widely among industries concerned with bacterial drug resistance and bacterial behavior.

For his scientific contributions Matin has received numerous awards and honors. These include his appointment as a Fulbright Scholar from 1964 until 1971, election to the American Academy of Microbiology, and inclusion in publications such as *Who's Who in the Frontiers of Science* and *Outstanding People of the 20th Century.*

See also Antibiotic resistance, tests for; Bacterial adaptation

MCCARTY, MACLYN (1911-)

American bacteriologist

Maclyn McCarty is a distinguished bacteriologist who has done important work on the biology of **Streptococci** and the origins of rheumatic fever, but he is best known for his involvement in early experiments which established the function of **DNA**. In collaboration with Oswald Avery and **Colin Munro MacLeod**, McCarty identified DNA as the substance which controls heredity in living cells. The three men published an article describing their experiment in 1944, and their work opened the way for further studies in bacteriological physiology, the most important of which was the demonstration of the chemical structure of DNA by James Watson and **Francis Crick** in 1953.

McCarty was born in South Bend, Indiana. His father worked for the Studebaker Corporation and the family moved often, with McCarty attending five schools in three different cities by the time he reached the sixth grade. In his autobiographical book, *The Transforming Principle,* McCarty recalled the experience as positive, believing that moving so often made him an inquisitive and alert child. He spent a year at Culver Academy in Indiana from 1925 to 1926, and he finished high school in Kenosha, Wisconsin. His family moved to Portland, Oregon, and McCarty attended Stanford University in California. He majored in **biochemistry** under James Murray Luck, who was then launching the *Annual Review of Biochemistry.* McCarty presented public seminars on topics derived from articles submitted to this publication, and he graduated with a B.A. in 1933.

Although Luck asked him to remain at Stanford, McCarty entered medical school at Johns Hopkins in Baltimore in 1933. He was married during medical school days, and he spent a summer of research at the Mayo Clinic in Minnesota. After graduation, McCarty spent three years working in pediatric medicine at the Johns Hopkins Hospital. Even in the decade before **penicillin**, new chemotherapeutic agents

had begun to change infectious disease therapy. McCarty treated children suffering from Pneumococcal **pneumonia**, and he was able to save a child suffering from a Streptococcal infection, then almost uniformly fatal, by the use of the newly available sulfonamide antibacterials. Both of these groups of **bacteria**, *Streptococcus* and the *Pneumococcus*, would play important roles throughout the remainder of McCarty's career.

McCarty spent his first full year of medical research at New York University in 1940, in the laboratory of W. S. Tillett. In 1941, McCarty was awarded a National Research Council grant, and Tillett recommended him for a position with Oswald Avery at the Rockefeller Institute, which was one of the most important centers of biomedical research in the United States. For many years, Avery had been working with Colin Munro MacLeod on Pneumococci. In 1928, the British microbiologist Frederick Griffith had discovered what he called a "transforming principle" in Pneumococci. In a series of experiments now considered a turning point in the history of genetics, Griffith had established that living individuals of one strain or variety of Pneumococci could be changed into another, with different characteristics, by the application of material taken from dead individuals of a second strain. When McCarty joined Avery and MacLeod, the chemical nature of this transforming material was not known, and this was what their experiments were designed to discover.

In an effort to determine the chemical nature of Griffith's transforming principle, McCarty began as more of a lab assistant than an equal partner. Avery and MacLeod had decided that the material belonged to one of two classes of organic compounds: it was either a protein or a nucleic acid. They were predisposed to think it was a protein, or possibly **RNA**, and their experimental work was based on efforts to selectively disable the ability of this material to transform strains of Pneumococci. Evidence that came to light during 1942 indicated that the material was not a protein but a nucleic acid, and it began to seem increasingly possible that DNA was the molecule for which they were searching. McCarty's most important contribution was the preparation of a deoxyribonuclease which disabled the transforming power of the material and established that it was DNA. They achieved these results by May of 1943, but Avery remained cautious, and their work was not published until 1944.

In 1946, McCarty was named head of a laboratory at the Rockefeller Institute which was dedicated to the study of the Streptococci. A relative of Pneumococci, Streptococci is a cause of rheumatic fever. McCarty's research established the important role played by the outer cellular covering of this bacteria. Using some of the same techniques he had used in his work on DNA, McCarty was able to isolate the cell wall of the Streptococcus and analyze its structure.

McCarty became a member of the Rockefeller Institute in 1950; he served as vice president of the institution from 1965 to 1978, and as physician in chief from 1965 to 1974. For his work as co-discoverer of the nature of the transforming principle, he won the Eli Lilly Award in Microbiology and **Immunology** in 1946 and was elected to the National Academy of Sciences in 1963. He won the first Waterford Biomedical Science Award of the Scripps Clinic and Research Foundation in 1977 and received honorary doctorates from Columbia University in 1976 and the University of Florida in 1977.

See also Microbial genetics; Microbiology, clinical; Streptococci and streptococcal infections

MEASLES

Measles is an infectious disease caused by a virus of the paramyxovirus group. It infects only man and the infection results in life-long **immunity** to the disease. It is one of several exanthematous (rash-producing) diseases of childhood, the others being rubella (German measles), chicken pox, and the now rare scarlet fever. The disease is particularly common in both pre-school and young school children.

The measles virus mainly infects mucous membranes of the respiratory tract and the skin. The symptoms include high fever, headache, hacking cough, conjunctivitis, and a rash that usually begins inside the mouth on the buccal mucosa as white spots, (called Koplik's spots) and progresses to a red rash that spreads to face, neck, trunk and extremities. The incubation period varies but is usually 10 to 12 days until symptoms appear. Four to five days before the onset of the rash, the child has fever or malaise and then may develop a sore throat and cough. The duration of the rash is usually five days. The child is infectious throughout the prodromal (early) period and for up to four days after the first appearance of the rash. The virus is highly contagious and is transmitted through respiratory droplets or though direct contact. Measles is also sometimes called rubeola or the nine-day measles.

Although certain complications can arise, in the vast majority of cases, children make a full recovery from measles. Acute local complications can occur if there is a secondary infection, for example **pneumonia** due to **bacteria** such as **staphylococci**, *Streptococcus pyogene*, pneumococci, or caused by the virus itself. Also, ear infections and secondary bacterial otitis media can seriously aggravate the disease. Central nervous system (CNS) complications include post-measles encephalitis, which occurs about 10 days after the illness with a significant mortality rate. Also, sub-acute sclerosing panencephalitis (SSPE), a rare fatal complication, presents several years after the original measles infection. Because hemorrhagic skin lesions, viraemia, and severe respiratory tract infection are particularly likely in malnourished infants, measles is still frequently a life-threatening infection in Africa and other underdeveloped regions of the world. The microbiological diagnosis of measles is not normally required because the symptoms are characteristic. However, if an acute CNS complication is suspected, paired sera are usually sent for the estimation of **complement** fixing antibodies to measles. If SSPE is suspected, the measles **antibody** titres in the CSF (determining the level of antibodies present) are also estimated.

Epidemiological studies have shown that there is a good correlation between the size of a population and the number of cases of measles. A population of at least 500,000 is required to provide sufficient susceptible individuals (i.e.

births) to maintain the virus within the population. Below that level, the virus will eventually die out unless it is re-introduced from an outside source. On the geological time-scale, man has evolved recently and has only existed in large populations in comparatively modern times. In the past, when human beings lived in small populations, it is concluded that the measles virus could not exist in its present form. It may have had another strategy of infection such as to persist in some form and infect the occasional susceptible passer-by, but this remains unproven. It has been suggested that the modern measles virus evolved from an ancestral animal virus, which is also common to the modern canine distemper and the cattle disease rinderpest. This theory is based on the similarities between these **viruses**, and on the fact that these animals have been commensal (living in close proximity) with man since his nomadic days. The ancestral virus is thought to have evolved into the modern measles virus when changes in the social behavior of man gave rise to populations large enough to maintain infection. This evolutionary event would have occurred within the last 6000 years when the river valley civilizations of the Tigris and Euphrates were established. To our knowledge, measles was first described as a disease in ninth century when a Persian physician, Rhazes, was the first to differentiate between measles and **smallpox**. The physician Rhazes also made the observation that the fever accompanying the disease is a bodily defense and not the disease itself. His writings on the subject were translated into English and published in 1847.

The measles virus itself was first discovered in 1930, and **John F. Enders** of the Children's Hospital in Boston successfully isolated the measles virus in 1954. Enders then began looking for an attenuated strain, which might be suitable for a live-virus **vaccine**. A successful **immunization** program for measles was begun soon after. Today measles is controlled in the United States with a **vaccination** that confers immunity against measles, **mumps**, and rubella and is commonly called the MMR vaccine. Following a series of measles **epidemics** occurring in the teenage population, a second MMR shot is now sometimes required by many school-age children as it was found that one vaccination appeared not to confer life-long immunity.

In October 1978, the Department of Health, Education, and Welfare announced their intention of eliminating the measles virus from the U.S.A. This idea was inspired by the apparently successful global elimination of smallpox by the **World Health Organization** vaccination program, which recorded its last smallpox case in 1977.

Death from measles due to respiratory or neurological causes occurs in about 1 out of every 1000 cases and encephalitis also occurs at this frequency, with survivors of the latter often having permanent brain damage. Measles virus meets all the currently held criteria for successful elimination. It only multiplies in man; there is a good live vaccine (95 % effective) and only one sero-type of the virus is known. Usually measles virus causes an acute infection but, rarely (1 out of every million cases), the virus persists and reappears some 2-6 years causing SSPE. However, measles virus can only be recovered with difficulty from infected tissue and SSPE is a non-transmissible disease. To successfully eliminate measles, it would be necessary to achieve a high immunization level, especially in children.

Measles rash on a child's back.

See also Antibody-antigen, biochemical and molecular reactions; History of immunology; History of public health; Immunity, active, passive and delayed; Immunology; Varicella; Viruses and responses to viral infection

MEDAWAR, PETER BRIAN (1915-1987)

English biologist

Peter Brian Medawar made major contributions to the study of **immunology** and was awarded the Nobel Prize in physiology or medicine in 1960. Working extensively with skin grafts, he and his collaborators proved that the **immune system** learns to distinguish between "self" and "non-self." During his career, Medawar also became a prolific author, penning books such as *The Uniqueness of the Individual* and *Advice to a Young Scientist.*

Medawar was born on February 28, 1915, in Rio de Janeiro, Brazil, to Nicholas Medawar and the former Edith Muriel Dowling. When he was a young boy, his family moved to England, which he thereafter called home. Medawar attended secondary school at Marlborough College, where he first became interested in biology. The biology master encouraged Medawar to pursue the science under the tutelage of one of his former students, John Young, at Magdalen College. Medawar followed this advice and enrolled at Magdalen in 1932 as a zoology student.

Medawar earned his bachelor's degree from Magdalen in 1935, the same year he accepted an appointment as Christopher Welch Scholar and Senior Demonstrator at Magdalen College. He followed Young's recommendation that he work with pathologist Howard Florey, who was undertaking a study of **penicillin**, work for which he would later become well known. Medawar leaned toward experimental embryology and tissue cultures. While at Magdalen, he met and married a fellow zoology student. Medawar and his wife had four children.

In 1938, Medawar, by examination, became a fellow of Magdalen College and received the Edward Chapman Research Prize. A year later, he received his master's from Oxford. When World War II broke out in Europe, the Medical Research Council asked Medawar to concentrate his research on tissue transplants, primarily skin grafts. While this took him away from his initial research studies into embryology, his work with the military would come to drive his future research and eventually lead to a Nobel Prize.

During the war, Medawar developed a concentrated form of fibrinogen, a component of the blood. This substance acted as a glue to reattach severed nerves, and found a place in the treatment of skin grafts and in other operations. More importantly to Medawar's future research, however, were his studies at the Burns Unit of the Glasgow Royal Infirmary in Scotland. His task was to determine why patients rejected donor skin grafts. He observed that the rejection time for donor grafts was noticeably longer for initial grafts, compared to those grafts that were transplanted for a second time. Medawar noted the similarity between this reaction and the body's reaction to an invading virus or **bacteria**. He formed the opinion that the body's rejection of skin grafts was immunological in nature; the body built up an **immunity** to the first graft and then called on that already-built-up immunity to quickly reject a second graft.

Upon his return from the Burns Unit to Oxford, he began his studies of immunology in the laboratory. In 1944, he became a senior research fellow of St. John's College, Oxford, and university demonstrator in zoology and comparative anatomy. Although he qualified for and passed his examinations for a doctorate in philosophy while at Oxford, Medawar opted against accepting it because it would cost more than he could afford. In his autobiography, *Memoir of a Thinking Radish,* he wrote, "The degree served no useful purpose and cost, I learned, as much as it cost in those days to have an appendectomy. Having just had the latter as a matter of urgency, I thought that to have both would border on self-indulgence, so I remained a plain mister until I became a prof." He continued as researcher at Oxford University through 1947.

During that year Medawar accepted an appointment as Mason professor of zoology at the University of Birmingham. He brought with him one of his best graduate students at Oxford, Rupert Everett "Bill" Billingham. Another graduate student, Leslie Brent, soon joined them and the three began what was to become a very productive collaboration that spanned several years. Their research progressed through Medawar's appointment as dean of science, through his several-month-long trip to the Rockefeller Institute in New York in 1949—the same year he received the title of fellow from the Royal Society—and even a relocation to another college. In 1951, Medawar accepted a position as Jodrell Professor of Zoology and Comparative Anatomy at University College, London. Billingham and Brent followed him.

Their most important discovery had its experimental root in a promise Medawar made at the International Congress of Genetics at Stockholm in 1948. He told another investigator, Hugh Donald, that he could formulate a foolproof method for distinguishing identical from fraternal twin calves. He and Billingham felt they could easily tell the twins apart by transplanting a skin graft from one twin to the other. They reasoned that a calf of an identical pair would accept a skin graft from its twin because the two originated from the same egg, whereas a calf would reject a graft from its fraternal twin because they came from two separate eggs. The results did not bear this out, however. The calves accepted skin grafts from their twins regardless of their status as identical or fraternal. Puzzled, they repeated the experiment, but received the same results.

They found their error when they became aware of work done by Dr. **Frank Macfarlane Burnet** of the University of Melbourne, and Ray D. Owen of the California Institute of Technology. Owen found that blood transfuses between twin calves, both fraternal and identical. Burnet believed that an individual's immunological framework developed before birth, and felt Owen's finding demonstrated this by showing that the immune system tolerates those tissues that are made known to it before a certain age. In other words, the body does not recognize donated tissue as alien if it has had some exposure to it at an early age. Burnet predicted that this immunological tolerance for non-native tissue could be reproduced in a lab. Medawar, Billingham, and Brent set out to test Burnet's hypothesis.

The three-scientist team worked closely together, inoculating embryos from mice of one strain with tissue cells from donor mice of another strain. When the mice had matured, the trio grafted skin from the donor mice to the inoculated mice. Normally, mice reject skin grafts from other mice, but the inoculated mice in their experiment accepted the donor skin grafts. They did not develop an immunological reaction. The prenatal encounter had given the inoculated mice an acquired immunological tolerance. They had proven Burnet's hypothesis. They published their findings in a 1953 article in *Nature*. Although their research had no applications to transplants among humans, it showed that transplants were possible.

In the years following publication of the research, Medawar accepted several honors, including the Royal Medal from the Royal Society in 1959. A year later, he and Burnet accepted the Nobel Prize for Physiology or Medicine for their discovery of acquired immunological tolerance: Burnet developed the theory and Medawar proved it. Medawar shared the prize money with Billingham and Brent.

Medawar's scientific concerns extended beyond immunology, even during the years of his work toward acquired immunological tolerance. While at Birmingham, he and Billingham also investigated pigment spread, a phenomenon seen in some guinea pigs and cattle where the dark spots spread into the light areas of the skin. "Thus if a dark skin graft were transplanted into the middle of a pale area of skin it would soon come to be surrounded by a progressively widening ring of dark skin," Medawar asserted in his autobiography. The team conducted a variety of experiments, hoping to show that the dark pigment cells were somehow "infecting" the pale pigment cells. The tests never panned out.

Medawar also delved into animal behavior at Birmingham. He edited a book on the subject by noted scientist Nikolaas Tinbergen, who ultimately netted a Nobel Prize in 1973. In 1957, Medawar also became a book author with his first offering, *The Uniqueness of the Individual,* which was actually a collection of essays. In 1959, his second book, *The Future of Man,* was issued, containing a compilation of a series of broadcasts he read for British Broadcasting Corporation (BBC) radio. The series examined the impacts of **evolution** on man.

Medawar remained at University College until 1962 when he took the post of director of the National Institute for Medical Research in London, where he continued his study of transplants and immunology. While there, he continued writing with mainly philosophical themes. *The Art of the Soluble,* published in 1967, is an assembly of essays, while his 1969 book, *Induction and Intuition in Scientific Thought,* is a sequence of lectures examining the thought processes of scientists. In 1969 Medawar, then president of the British Association for the Advancement of Science, experienced the first of a series of strokes while speaking at the group's annual meeting. He finally retired from his position as director of the National Institute for Medical Research in 1971. In spite of his physical limitations, he went ahead with scientific research in his lab at the clinical research center of the Medical Research Council. There he began studying cancer.

Through the 1970s and 1980s, Medawar produced several other books—some with his wife as co-author—in addition to his many essays on growth, aging, immunity, and cellular transformations. In one of his most well-known books, *Advice to a Young Scientist,* Medawar asserted that for scientists, curiosity was more important that genius.

See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Antibody, monoclonal; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunochemistry; Immunogenetics; Major histocompatibility complex (MHC); Transplantation genetics and immunology

MEDICAL TRAINING AND CAREERS IN MICROBIOLOGY

The world of microbiology overlaps the world of medicine. As a result, trained microbiologists find a diversity of career paths and opportunity in medicine.

Research in medical microbiology can involve clinical or basic science. **Clinical microbiology** focuses on the microbiological basis of various diseases and how to alleviate the suffering caused by the infectious microorganism. Basic medical research is concerned more with the molecular events associated with infectious diseases or illnesses.

Both medical training and microbiology contain many different areas of study. Medical microbiology is likewise an area of many specialties. A medical bacteriologist can study how **bacteria** can infect humans and cause disease, and how these disease processes can be dealt with. A medical mycologist can study pathogenic (disease-causing) **fungi**, molds and **yeast** to find out how they cause disease. A parasitologist is concerned with how parasitic **microorganisms** (those that require a host in order to live) cause disease. A medical virologist can study the diseases attributed to infection by a virus, such as the hemorrhagic fever caused by the **Ebola virus**.

The paths to these varied disciplines of study are also varied. One route that a student can take to incorporate both research training and medical education is the combined M.D.-PhD. program. In several years of rigorous study, students become physician-scientists. Often, graduates develop a clinical practice combined with basic research. The experience gained at the bedside can provide research ideas. Conversely, laboratory techniques can be brought to bear on unraveling the basis of human disease. The M.D.–PhD. training exemplifies what is known as the transdisciplinary approach. Incorporating different approaches to an issue can suggest treatment or research strategies that might otherwise not be evident if an issue were addressed from only one perspective.

The training for a career in the area of medicine and medical microbiology begins in high school. Courses in the sciences lay the foundation for the more in-depth training that will follow in university or technical institution. With undergraduate level training, career paths can include research assistant, providing key technical support to a research team, quality assurance in the food, industrial or environmental microbiology areas, and medical technology.

Medical microbiology training at the undergraduate and graduate levels, in the absence of simultaneous medical training, can also lead to a career as a clinical microbiologist. Such scientists are employed in universities, hospitals and in the public sector. For example, the United Kingdom has an extensive Public Health Laboratory Service. The PHLS employs clinical microbiologists in reference laboratories, to develop or augment test methods, and as epidemiologists. The latter are involved in determining the underlying causes of disease outbreaks and in uncovering potential microbiological health threats. Training in medical microbiology can be at the Baccalaureate level, and in research that leads to a Masters or a Doctoral degree. The latter is usually undertaken if the aim

Working as a specialist in a medical microbiology laboratory is one of many careers available in the field.

is to do original and independent research, teach undergraduate and graduate students, or to assume an executive position.

Medical technologists are involved in carrying out the myriad of microbiological tests that are performed on samples such as urine, blood and other body fluids to distinguish pathogenic microorganisms from the normal flora of the body. This can be very much akin to detective work, involving the testing of samples by various means to resolve he identity of an organism based on the various biochemical behaviors. Increasingly, such work is done in conjunction with automated equipment. Medical technologists must be skilled at scheduling tests efficiently, independently and as part of a team. Training as a medical technologist is typically at a community college or technical institution and usually requires two years.

As in the other disciplines of medical microbiology, medical technology is a specialized field. Histopathology is the examination of body cells or tissues to detect or rule out disease. This speciality involves knowledge of light and **electron microscopic examination** of samples. Cytology is the study of cells for abnormalities that might be indicative of infection or other malady, such as cancer. Medical **immunology** studies the response of the host to infection. A medical immunologist is skilled at identifying those immune cells that active in combating an infection. Medical technology also encompasses the area of clinical **biochemistry**, where cells and body fluids are analyzed for the presence of components related to disease. Of course the study of microorganism involvement in disease requires medical technologists who are specialized microbiologists and virologists, as two examples.

Medical microbiologists also can find a rewarding career path in industry. Specifically, the knowledge of the susceptibility or resistance of microorganisms to antimicrobial

drugs is crucial to the development of new drugs. Work can be at the research and development level, in the manufacture of drugs, in the regulation and licensing of new antimicrobial agents, and even in the sale of drugs. For example, the sale of a product can be facilitated by the interaction of the sales associate and physician client on an equal footing in terms of knowledge of antimicrobial therapy or disease processes.

Following the acquisition of a graduate or medical degree, specialization in a chosen area can involve years of post-graduate or medical residence. The road to a university lab or the operating room requires dedication and over a decade of intensive study.

Careers in medical science and medical microbiology need not be focused at the patient bedside or at the lab bench. Increasingly, the medical and infectious disease fields are benefiting from the advice of consultants and those who are able to direct programs. Medical or microbiological training combined with experience or training in areas such as law or business administration present an attractive career combination.

See also Bioinformatics and computational biology; Food safety; History of public health; Hygiene; World Health Organization

MEMBRANE FLUIDITY

The membranes of **bacteria** function to give the bacterium its shape, allow the passage of molecules from the outside in and from the inside out, and to prevent the internal contents from leaking out. Gram-negative bacteria have two membranes that make up their cell wall, whereas Gram-positive bacteria have a single membrane as a component of their cell wall. Yeasts and **fungi** have another specialized nuclear membrane that compartmentalizes the genetic material of the cell.

For all these functions, the membrane must be fluid. For example, if the interior of a bacterial membrane was crystalline, the movement of molecules across the membrane would be extremely difficult and the bacterium would not survive.

Membrane fluidity is assured by the construction of a typical membrane. This construction can be described by the fluid mosaic model. The mosaic consists of objects, such as proteins, which are embedded in a supporting—but mobile—structure of lipid.

The fluid mosaic model for membrane construction was proposed in 1972 by S. J. Singer of the University of California at San Diego and G. L. Nicolson of the Salk Institute. Since that time, the evidence in support of a fluid membrane has become irrefutable.

In a fluid membrane, proteins may be exposed on the inner surface of the membrane, the outer surface, or at both surfaces. Depending on their association with neighbouring molecules, the proteins may be held in place or may capable of a slow drifting movement within the membrane. Some proteins associate together to form pores through which molecules can pass in a regulated fashion (such as by the charge or size of the molecule).

The fluid nature of the membrane rest with the supporting structure of the lipids. Membrane lipids of **microorganisms** tend to be a type of lipid termed phospholipid. A phospholipid consists of fatty acid chains that terminate at one end in a phosphate group. The fatty acid chains are not charged, and so do not tend to associate with water. In other words they are **hydrophobic**. On the other hand, the charged phosphate head group does tend to associate with water. In other words they are hydrophilic. The way to reconcile these chemistry differences in the membrane are to orient the **phospholipids** with the water-phobic tails pointing inside and the water-phyllic heads oriented to the watery external environment. This creates two so-called leaflets, or a bilayer, of phospholipid. Essentially the membrane is a two dimensional fluid that is made mostly of phospholipids. The consistency of the membrane is about that of olive oil.

Regions of the membrane will consist solely of the lipid bilayer. Molecules that are more hydrophobic will tend to dissolve into these regions, and so can move across the membrane passively. Additionally, some of the proteins embedded in the bilayer will have a transport function, to actively pump or move molecules across the membrane.

The fluidity of microbial membranes also allows the constituent proteins to adopt new configurations, as happens when molecules bind to receptor portions of the protein. These configurational changes are an important mechanism of signaling other proteins and initiating a response to, for example, the presence of a food source. For example, a protein that binds a molecule may rotate, carrying the molecule across the membrane and releasing the molecule on the other side. In bacteria, the membrane proteins tend to be located more in one leaflet of the membrane than the other. This asymmetric arrangement largely drives the various transport and other functions that the membrane can perform.

The phospholipids are capable of a drifting movement laterally on whatever side of the membrane they happen to be. Measurements of this movement have shown that the drifting can actually be quite rapid. A flip-flop motion across to the other side of the membrane is rare. The fluid motion of the phospholipids increases if the hydrophobic tail portion contains more double bonds, which cause the tail to be kinked instead of straight. Such alteration of the phospholipid tails can occur in response to temperature change. For example if the temperature decreases, a bacterium may alter the phospholipid chemistry so as to increase the fluidity of the membrane.

See also Bacterial membranes and cell wall

MEMBRANE TRANSPORT, EUKARYOTIC •

see CELL MEMBRANE TRANSPORT

MEMBRANE TRANSPORT, PROKARYOTIC •

see PROKARYOTIC MEMBRANE TRANSPORT

MENINGITIS, BACTERIAL AND VIRAL

Meningitis is a potentially fatal **inflammation** of the meninges, the thin, membranous covering of the brain and the spinal cord. Meningitis is most commonly caused by infection (by **bacteria**, **viruses**, or **fungi**), although it can also be caused by bleeding into the meninges, cancer, or diseases of the **immune system**.

The meninges are three separate membranes, layered together, which serve to encase the brain and spinal cord. The dura is the toughest, outermost layer, and is closely attached to the inside of the skull. The middle layer, the arachnoid, is important in the normal flow of the cerebrospinal fluid (CSF), a lubricating fluid that bathes both the brain and the spinal cord. The innermost layer, the pia, helps direct brain blood vessels into the brain. The space between the arachnoid and the pia contains CSF, which serves to help insulate the brain from trauma. Through this space course many blood vessels. CSF, produced within specialized chambers deep inside the brain, flows over the surface of the brain and spinal cord. This fluid serves to cushion these relatively delicate structures, as well as supplying important nutrients for brain cells. CSF is reabsorbed by blood vessels that are located within the meninges.

The cells lining the brain's capillaries (tiny blood vessels) are specifically designed to prevent many substances from passing into brain tissue. This is commonly referred to as the blood-brain barrier. The blood-brain barrier prevents various toxins (substances which could be poisonous to brain tissue), as well as many agents of infection, from crossing from the blood stream into the brain tissue. While this barrier obviously is an important protective feature for the brain, it also serves to complicate therapy in the case of an infection, by making it difficult for medications to pass out of the blood and into the brain tissue where the infection resides.

The most common infectious causes of meningitis vary according to an individual host's age, habits and living environment, and health status. In newborns, the most common agents of meningitis are those that are contracted from the newborn's mother, including Group B *Streptococci* (becoming an increasingly common infecting organism in the newborn period), *Escherichia coli,* and *Listeria monocytogenes*. Older children are more frequently infected by *Haemophilus influenzae, Neisseria meningitidis,* and *Streptococcus pneumoniae,* while adults are infected by *S. pneumoniae* and *N. meningitidis*. *N. meningitidis* is the only organism that can cause **epidemics** of meningitis. These have occurred in particular when a child in a crowded day-care situation, a college student in a dormitory, or a military recruit in a crowded training camp has fallen ill with *N. meningitidis* meningitis.

Viral causes of meningitis include the **herpes** simplex viruses, **mumps** and **measles** viruses (against which most children are protected due to mass **immunization** programs), the virus that causes chicken pox, the **rabies** virus, and a number of viruses that are acquired through the bite of infected mosquitoes. Patients with **AIDS** (Acquired Immune Deficiency Syndrome) are more susceptible to certain infectious causes of meningitis, including by certain fungal agents, as well as by the agent that causes **tuberculosis**. Patients who have had their spleens removed, or whose spleens are no longer functional (as in the case of patients with sickle cell disease) are more susceptible to certain infections, including those caused by *N. meningitidis and S. pneumoniae*.

The majority of meningitis infections are acquired by blood-borne spread. An individual may have another type of infection (of the lungs, throat, or tissues of the heart) caused by an organism that can also cause meningitis. The organism multiplies, finds its way into the blood stream, and is delivered in sufficient quantities to invade past the blood-brain barrier.

Direct spread occurs when an already resident infectious agent spreads from infected tissue next to or very near the meninges, for example from an ear or sinus infection. Patients who suffer from skull fractures provide openings to the sinuses, nasal passages, and middle ears. Organisms that frequently live in the human respiratory system can then pass through these openings to reach the meninges and cause infection. Similarly, patients who undergo surgical procedures or who have had foreign bodies surgically placed within their skulls (such as tubes to drain abnormal amounts of accumulated CSF) have an increased risk of the organisms causing meningitis being introduced to the meninges.

The most classic symptoms of meningitis (particularly of bacterial meningitis) include fever, headache, vomiting, photophobia (sensitivity to light), irritability, lethargy (severe fatigue), and stiff neck. The disease progresses with seizures, confusion, and eventually coma.

Damage due to meningitis occurs from a variety of phenomena. The action of infectious agents on the brain tissue is one direct cause of damage. Other types of damage may be due to mechanical effects of swelling of brain tissue, and compression against the bony surface of the skull. Swelling of the meninges may interfere with the normal absorption of CSF by blood vessels, causing accumulation of CSF and damage due to resulting pressure on the brain. Interference with the brain's carefully regulated chemical environment may cause damaging amounts of normally present substances (carbon dioxide, potassium) to accumulate. Inflammation may cause the blood-brain barrier to become less effective at preventing the passage of toxic substances into brain tissue.

Antibiotic medications (forms of penicillins and cephalosporins, for example) are the most important element of treatment against bacterial agents of meningitis. Because of the effectiveness of the blood-brain barrier in preventing passage of substances into the brain, medications must be delivered directly into the patient's veins (intravenous or IV) at very high doses. Antiviral medications (acyclovir) may be helpful in the case of viral meningitis, and antifungal medications are available as well.

Other treatment for meningitis involves decreasing inflammation (with steroid preparations) and paying careful attention to the balance of fluids, glucose, sodium, potassium, oxygen, and carbon dioxide in the patient's system. Patients who develop seizures will require medications to halt the seizures and prevent their return.

A series of immunizations against *Haemophilus influenzae,* started at two months of age, has greatly reduced the inci-

dence of that form of meningitis. Vaccines also exist against *Neisseria meningitidis* and *Streptococcus pneumoniae* bacteria, but these vaccines are only recommended for those people who have particular susceptibility to those organisms, due to certain immune deficiencies, lack of a spleen, or sickle cell anemia.

Because *N. meningitidis* is known to cause epidemics of disease, close contacts of patients with such meningitis are treated prophylactically, often with the antibiotic Rifampin. This measure generally prevents spread of the disease.

See also Bacteria and bacterial infection; Viruses and responses to viral infection

MESELSON, MATTHEW STANLEY (1930-)

American molecular biologist

Matthew Meselson, in collaboration with biologist Franklin W. Stahl, showed experimentally that the replication of **deoxyribonucleic acid** (**DNA**) in **bacteria** is semiconservative. Semiconservative replication occurs in a double stranded DNA molecule when the two strands are separated and a new strand is copied from the parental strand to produce two new double stranded DNA molecules. The new double stranded DNA molecule is semiconservative because only one strand is conserved from the parent; the other strand is a new copy. (Conservative replication occurs when one offspring of a molecule contains both parent strands and the other molecule offspring contains newly replicated strands) The classical experiment revealing semiconservative replication in bacteria was central to the understanding of the living cell and to modern **molecular biology**.

Matthew Stanley Meselson was born May 24, 1930, in Denver, Colorado. After graduating in 1951 with a Ph.D. in liberal arts from the University of Chicago, he continued his education with graduate studies at the California Institute of Technology in the field of chemistry. Meselson graduated with a Ph.D. in 1957, and remained at Cal Tech as a research fellow. He acquired the position of assistant professor of chemistry at Cal Tech in 1958. In 1960, Meselson moved to Cambridge, Massachusetts to fill the position of associate professor of natural sciences at Harvard University. In 1964, he was awarded professor of biology, which he held until 1976. He was appointed the title of Thomas Dudley Cabot professor of natural sciences in 1976. From that time on, Meselson held a concurrent appointment on the council of the Smithsonian Institute in Washington, DC.

After graduating from the University of Chicago, Meselson continued his education in chemistry at the California Institute of Technology. It was during his final year at Cal Tech that Meselson collaborated with Franklin Stahl on the classical experiment of semiconservative replication of DNA. Meselson and Stahl wanted to design and perform an experiment that would show the nature of DNA replication from parent to offspring using the **bacteriophage** T4 (a virus that destroys other cells, also called a phage). The idea was to use an isotope to mark the cells and centrifuge to separate particles that could be identified by their DNA and measure changes in the new generations of DNA. Meselson, Stahl, and Jerome Vinograd originally designed this technique of isolating phage samples. The phage samples isolated would contain various amounts of the isotope based on the rate of DNA replication. The amount of isotope incorporated in the new DNA strands, they hoped, would be large enough to determine quantitatively. The experiments, however, were not successful. After further contemplation, Meselson and Stahl decided to abandon the use of bacteriophage T4 and the isotope and use instead the bacteria ***Escherichia coli*** (*E. coli*) and the heavy nitrogen isotope 15N as the labeling substance. This time when the same experimental steps were repeated, the analysis showed three distinct types of bacterial DNA, two from the original parent strands and one from the offspring. Analysis of this offspring showed each strand of DNA came from a different parent. Thus the theory of semiconservative replication of DNA had been proven. With this notable start to his scientific career Meselson embarked on another collaboration, this time with biologists **Sydney Brenner**, from the Medical Research Council's Division of Molecular Biology in Cambridge, England, and **François Jacob** from the Pasteur Institute Laboratories in Paris, France. Together, Meselson, Brenner, and Jacob performed a series of experiments in which they showed that when the bacteriophage T4 enters a bacterial cell, the phage DNA incorporates into the cellular DNA and causes the release of messenger **RNA**. Messenger RNA instructs the cell to manufacture phage proteins instead of the bacterial cell proteins that are normally produced. These experiments led to the discovery of the role of messenger RNA as the instructions that the bacterial cell reads to produce the desired protein products. These experiments also showed that the bacterial cell could produce proteins from messenger RNA that are not native to the cell in which it occurs.

In his own laboratory at Harvard University, Meselson and a postdoctoral fellow, Robert Yuan, were developing and purifying one of the first of many known **restriction enzymes** commonly used in molecular biological analyses. Restriction **enzymes** are developed by cultivating bacterial strains with phages. Bacterial strains that have the ability to restrict foreign DNA produce a protein called an enzyme that actually chews up or degrades the foreign DNA. This enzyme is able to break up the foreign DNA sequences into a number of small segments by breaking the double stranded DNA at particular locations. Purification of these enzymes allowed mapping of various DNA sequences to be accomplished. The use of purified restriction enzymes became a common practice in the field of molecular biology to map and determine contents of many DNA sequences.

After many years working with the bacteria *E. coli*, Meselson decided to investigate the fundamentals of DNA replication and repair in other organisms. He chose to work on the fruit fly called *Drosophila melanogaster*. Meselson discovered that the fruit fly contained particular DNA sequences that would be transcribed only when induced by heat shock or stress conditions. These particular heat shock genes required a

specific setup of DNA bases upstream of the initiation site in order for **transcription** to occur. If the number of bases was increased or reduced from what was required, the **gene** would not be transcribed. Meselson also found that there were particular DNA sequences that could be recombined or moved around within the entire chromosome of DNA. These moveable segments are termed **transposons**. Transposons, when inserted into particular sites within the sequence, can either turn on or turn off expression of the gene that is near it, causing **mutations** within the fly. These studies contributed to the identity of particular regulatory and structural features of the fruit fly as well as to the overall understanding of the properties of DNA.

Throughout his career as a scientist, Meselson has written over 50 papers published in major scientific journals and received many honors and awards for his contributions to the field of molecular biology. In 1963, Meselson received the National Academy of Science Prize for Molecular Biology, followed by the Eli Lilly Award for Microbiology and **Immunology** in 1964. He was awarded the Lehman Award in 1975 and the Presidential award in 1983, both from the New York Academy of Sciences. In 1990, Meselson received the Science Freedom and Responsibility Award from the American Association for the Advancement of Science. Meselson has also delved into political issues, particularly on government proposals for worldwide chemical and biological weapon disarmament.

See also Microbial genetics; Transposition

MESOPHILIC BACTERIA

Mesophiles are **microorganisms** such as some species of **Bacteria**, **Fungi**, and even some **Archaea** that are best active at median temperatures. For instance, bacterial species involved in biodegradation (i.e., digestion and decomposition of organic matter), which are more active in temperatures ranging from approximately 70° - 90°F (approx. 15°–40°C), are termed mesophilic bacteria. They take part in the web of micro-organic activity that form the humus layer in forests and other fertile soils, by decomposing both vegetable and animal matter.

At the beginning of the decomposition process, another group of bacteria, psychrophylic bacteria, start the process because they are active in lower temperatures up to 55°F (from below zero up to 20°C), and generate heat in the process. When the temperature inside the decomposing layer reaches 50–100°F, it attracts mesophilic bacteria to continue the biodegradation. The peak of reproductive and activity of mesophilic bacteria is reached between 86–99°F (30–37°C), and further increases the temperature in the soil environment. Between 104–170°F (40–85°C, or even higher), another group of bacteria (thermophyllic bacteria) takes up the process that will eventually result in organic soil, or humus. Several species of fungi also take part in each decomposing step.

Mesophilic bacteria are also involved in food **contamination** and degradation, such as in bread, grains, dairies, and meats. Examples of common mesophilic bacteria are *Listeria monocytogenes, Pesudomonas maltophilia, Thiobacillus novellus, Staphylococcus aureus, Streptococcus pyrogenes, Streptococcus pneumoniae, Escherichia coli, and Clostridium kluyveri*. Bacterial infections in humans are mostly caused by mesophilic bacteria that find their optimum growth temperature around 37°C (98.6°F), the normal human body temperature. Beneficial bacteria found in human intestinal flora are also mesophiles, such as dietary *Lactobacillus acidophilus*.

See also Archaeobacteria; Bacteria and bacterial infection; Biodegradable substances; Composting, microbiological aspects; Extremophiles

METABOLISM

Metabolism is the sum total of chemical changes that occur in living organisms and which are fundamental to life. All prokaryotic and eukaryotic cells are metabolically active. The sole exception is **viruses**, but even viruses require a metabolically active host for their replication.

Metabolism involves the use of compounds. Nutrients from the environment are used in two ways by **microorganisms**. They can be the building blocks of various components of the microorganism (assimilation or anabolism). Or, nutrients can be degraded to yield energy (dissimilation or catabolism). Some so-called amphibolic biochemical pathways can serve both purposes. The continual processes of breakdown and re-synthesis are in a balance that is referred to as turnover. Metabolism is an open system. That is, there are constant inputs and outputs. A chain of metabolic reactions is said to be in a steady state when the concentration of all intermediates remains constant, despite the net flow of material through the system. That means the concentration of intermediates remains constant, while a product is formed at the expense of the substrate.

Primary metabolism comprises those metabolic processes that are basically similar in all living cells and are necessary for cellular maintenance and survival. They include the fundamental processes of growth (e.g., the synthesis of biopolymers and the macromolecular structures of cells and organelles), energy production (glycolysis and the tricarboxylic acid cycle) and the turnover of cell constituents. Secondary metabolism refers to the production of substances, such as bile pigments from porphyrins in humans, which only occur in certain eukaryotic tissues and are distinct from the primary metabolic pathways.

Metabolic control processes that occur inside cells include regulation of **gene** expression and metabolic feedback or feed-forward processes. The triggers of differential gene expression may be chemical, physical (e.g., bacterial cell density), or environmental (e.g., light). Differential gene expression is responsible for the regulation, at the molecular level, of differentiation and development, as well as the maintenance of numerous cellular "house-keeping" reactions, which are essential for the day-to-day functioning of a microorganism. In many metabolic pathways, the metabolites (substances produced or consumed by metabolism) themselves can act

directly as signals in the control of their own breakdown and synthesis. Feedback control can be negative or positive. Negative feedback results in the inhibition by an end product, of the activity or synthesis of an enzyme or several **enzymes** in a reaction chain. The inhibition of the synthesis of enzymes is called enzyme repression. Inhibition of the activity of an enzyme by an end product is an allosteric effect and this type of feedback control is well known in many metabolic pathways (e.g., lactose **operon**). In positive feedback, an endproduct activates an enzyme responsible for its own production.

Many reactions in metabolism are cyclic. A metabolic cycle is a catalytic series of reactions, in which the product of one bimolecular (involving two molecules) reaction is regenerated as follows: $A + B \rightarrow C + A$. Thus, A acts catalytically and is required only in small amounts and A can be regarded as carrier of B. The catalytic function of A and other members of the metabolic cycle ensure economic conversion of B to C. B is the substrate of the metabolic cycle and C is the product. If intermediates are withdrawn from the metabolic cycle, e.g., for biosynthesis, the stationary concentrations of the metabolic cycle intermediates must be maintained by synthesis. Replenishment of depleted metabolic cycle intermediates is called anaplerosis. Anaplerosis may be served by a single reaction, which converts a common metabolite into an intermediate of the metabolic cycle. An example of this is pyruvate to oxaloacetate reaction in the tricarboxylic acid cycle. Alternatively, it may involve a metabolic sequence of reactions, i.e., an anaplerotic sequence. An example of this is the glycerate pathway which provides phosphoenol pyruvate for anaplerosis of the tricarboxylic acid cycle.

Prokaryotes exhibit a great diversity of metabolic options, even in a single organism. For example, ***Escherichia coli*** can produce energy by **respiration** or **fermentation**. Respiration can be under aerobic conditions (e.g., using O_2 as the final electron acceptor) or anaerobically (e.g., using something other than oxygen as the final electron acceptor). Compounds like lactose or glucose can be used as the only source of carbon. Other **bacteria** have other metabolic capabilities including the use of sunlight for energy.

Some of these mechanisms are also utilized by eukaryotic cells. In addition, prokaryotes have a number of energy-generating mechanisms that do not exist in eukaryotic cells. Prokaryotic fermentation can be uniquely done via the phosphoketolase and Enter-Doudoroff pathways. Anaerobic respiration is unique to prokaryotes, as is the use of inorganic compounds as energy sources or as carbon sources during bacterial **photosynthesis**. Archaebacteria possess metabolic pathways that use H_2 as the energy source with the production of methane, and a nonphotosynthetic metabolism that can convert light energy into chemical energy.

In bacteria, metabolic processes are coupled to the synthesis of adenosine triphosphate (ATP), the principle fuel source of the cell, through a series of membrane-bound proteins that constituent the **electron transport system**. The movement of protons from the inside to the outside of the membrane during the operation of the electron transport system can be used to drive many processes in a bacterium, such as the movement of the flagella used to power the bacterium along, and the synthesis of ATP in the process called oxidative phosphorylation.

The fermentative metabolism that is unique to some bacteria is evolutionarily ancient. This is consistent with the early appearance of bacteria on Earth, relative to eukaryotic organisms. But bacteria can also ferment sugars in the same way that brewing **yeast** (i.e., *Saccharomyces cerevesiae* ferment sugars to produce ethanol and carbon dioxide. This fermentation, via the so-called Embden Myerhoff pathway, can lead to different ends products in bacteria, such as lactic acid (e.g., *Lactobacillus*), a mixture of acids (*Enterobacteriacaeae*, butanediol (e.g., *Klebsiella*, and propionic acid (e.g., *Propionibacterium*).

See also Bacterial growth and division; Biochemistry

METCHNIKOFF, ÉLIE (1845-1916)

Russian immunologist

Élie Metchnikoff was a pioneer in the field of **immunology** and won the 1908 Nobel Prize in physiology or medicine for his discoveries of how the body protects itself from disease-causing organisms. Later in life, he became interested in the effects of nutrition on aging and health, which led him to advocate some controversial diet practices.

Metchnikoff, the youngest of five children, was born in the Ukrainian village of Ivanovka on May 16, 1845, to Emilia Nevahovna, daughter of a wealthy writer, and Ilya Ivanovich, an officer of the Imperial Guard in St. Petersburg. He enrolled at the Kharkov Lycee in 1856, where he developed an especially strong interest in biology. At age 16, he published a paper in a Moscow journal criticizing a geology textbook. After graduating from secondary school in 1862, he entered the University of Kharkov, where he completed a four-year program in two years. He also became an advocate of the theory of **evolution** by natural **selection** after reading Charles Darwin's *On the Origin of Species by Means of Natural Selection.*

In 1864, Metchnikoff traveled to Germany to study, where his work with nematodes (a species of worm) led to the surprising conclusion that the organism alternates between sexual and asexual generations. His studies at Kharkov, coupled with his interest in Darwin's theory, convinced him that highly evolved animals should show structural similarities to more primitive animals. He pursued his studies of invertebrates in Naples, Italy, where he collaborated with Russian zoologist Alexander Kovalevsky. They demonstrated the homology (similarity of structure) between the germ layers—embryonic sheets of cells that give rise to specific tissue—in different multicellular animals. For this work, the scientists were awarded the Karl Ernst von Baer Prize.

Metchnikoff was only twenty-two when he received the prize and had a promising career ahead of himself. However, he soon developed severe eye strain, a condition that hampered his work and prevented him from using the **microscope** for the next fifteen years. Nevertheless, in 1867, he completed his doctorate at the University of St. Petersburg with a thesis

on the embryonic development of fish and crustaceans. He taught at the university for the next six years before moving to the University of Odessa on the Black Sea where he studied marine animals.

During the summer of 1880, he spent a vacation on a farm where a beetle infection was destroying crops. In an attempt to curtail the devastation, Metchnikoff injected a fungus from a dead fly into a beetle to see if he could kill the pest. Metchnikoff carried this interest in infection with him when he left Odessa for Italy, following the assassination of Czar Alexander II in 1884. A zoologist up to that point, Metchnikoff began to focus more on pathology, or the study of diseases.

This **transformation** was due primarily to his study of the larva of the Bipinniara starfish. While studying this larva, which is transparent and can be easily observed under the microscope, Metchnikoff saw special cells surrounding and engulfing foreign bodies, similar to the actions of white blood cells in humans that were present in areas of **inflammation**. During a similar study of the water flea *Daphniae,* he observed white blood cells attacking needle-shaped spores that had invaded the insect's body. He called these cells phagocytes, from the Greek word *phagein,* meaning, to eat.

While scientists thought that human phagocytes merely transported foreign material throughout the body, and therefore spread disease, Metchnikoff realized they performed a protective function. He recognized that the human white blood cells and the starfish phagocytes were embryologically homologous, both being derived from the mesoderm layer of cells. He concluded that the human cells cleared the body of disease-causing organisms. In 1884, he injected infected blood under the skin of a frog and demonstrated that white blood cells in higher animals served a similar function as those in starfish larvae. The scientific community, however, still did not accept his idea that phagocytic cells fought off infections.

Metchnikoff returned to Odessa in 1886 and became the director of the Bacteriological Institute. He continued his research on phagocytes in animals and pursued vaccines for chicken cholera and sheep **anthrax**. Hounded by scientists and the press because of his lack of medical training, Metchnikoff fled Russia a year later. A chance meeting with French scientist **Louis Pasteur** led to a position as the director of a new laboratory at the Pasteur Institute in Paris. There, he continued his study of **phagocytosis** for the next twenty-eight years.

But conflict with his fellow scientists continued to follow him. Many scientists asserted that antibodies triggered the body's immune response to infection. Metchnikoff accepted the existence of antibodies but insisted that phagocytic cells represented another important arm of the **immune system**. His work at the Pasteur Institute led to many fundamental discoveries about the immune response, and one of his students, **Jules Bordet**, contributed important insights into the nature of **complement**, a system of antimicrobial **enzymes** triggered by antibodies. Metchnikoff received the Nobel Prize for physiology and medicine in 1908 jointly with **Paul Ehrlich** for their work in initiating the study of immunology and greatly influencing its development.

Metchnikoff's interest in **immunity** led to writings on aging and death. His book *The Nature of Man,* published in 1903, extolled the health virtues of "right living," which for him included consuming large amounts of fermented milk or yogurt made with a Bulgarian bacillus. In fact, his own name became associated with a popular commercial preparation of yogurt, although he received no royalties. With the exception of yogurt, Metchnikoff warned of eating uncooked foods, claiming that the **bacteria** present on them could cause cancer. Metchnikoff claimed he even plunged bananas into boiling water after unpeeling them and passed his silverware through flames before using it.

On July 15, 1916, after a series of heart attacks, Metchnikoff died in Paris at the age of 71. He was a member of the French Academy of Medicine, the Swedish Medical Society, and the Royal Society of London, from which he received the Copley Medal. He also received an honorary doctorate from Cambridge University.

See also Phagocyte and phagocytosis

METHANE OXIDIZING AND PRODUCING BACTERIA

Methane is a chemical compound that consists of a carbon atom to which are bound four hydrogen atoms. The gas is a major constituent of oxygen-free mud and water, marshes, the rumen of cattle and other animals, and the intestinal tract of mammals. In oxygen-free (anaerobic) environments, methane can be produced by a type of **bacteria** known as methanogenic bacteria. Methane can also be used as an energy source by other bacteria that grow in the presence of oxygen (aerobic bacteria), which break down the compound into carbon dioxide and water. These bacteria are known as methane oxidizing bacteria.

Bacteria from a number of genera are able to oxidize methane. These include *Methylosinus*, *Methylocystis*, *Methanomonas*, *Methylomonas*, *Methanobacter*, and *Methylococcus*. A characteristic feature of methane-oxidizing bacteria is the presence of an extensive system of membranes inside the bacterial cell. The membranes house the **enzymes** and other biochemical machinery needed to deal with the se of methane as an energy source.

The oxidation of methane by bacteria requires oxygen. The end result is the production of carbon dioxide and water. Methane oxidation is restricted to prokaryotes. Eukaryotic **microorganisms** such as algae and **fungi** do not oxidize methane.

The production of methane is a feature of anaerobic bacteria. Examples of methane producing genera are *Methanobacterium*, *Methanosarcina*, *Methanococcus*, and *Methanospirillum*. Methanogenic bacteria are widespread in nature, and are found in mud, sewage, and sludge and in the rumen of sheep and cattle. Some methanogenic bacteria have adapted to live in extreme environments. For example, *Methanococcus jannaschii* has an optimum growth temperature of 85° C (185° F), which is achieved in hot springs and thermal vents in the ocean. Such anaerobic bacteria are among

the oldest life forms on Earth. They evolved long before the presence of photosynthetic green plants, and so existed in an oxygen-free world.

In the rumen, the methane-producing bacteria occupy a central role in regulating the anaerobic breakdown (**fermentation**) of food. The bacteria remove hydrogen gas through the se of the gas in the reduction of carbon dioxide to form methane. By producing methane, the concentration of hydrogen is kept at a low level that allows other bacterial species to grow. This microbial diversity makes fermentation more efficient.

The bacterial production of methane is of economic importance. "Biogas" obtained from digesters can be a commercial and domestic energy source, although more economic sources of energy currently limit this use. In large-scale livestock operations, the use of methane producing bacteria is being increasing popular as a means of odor-control.

As on Earth, methane producing bacteria may be one of the earliest forms of life on other planets. Experiments that duplicate the atmosphere of the planet Mars have been successful in growing methane producing bacteria. Aside from its fundamental scientific importance, the discovery might be exploited in future manned missions to Mars. Methane is described as being a greenhouse gas, which means it can warm the surface atmosphere. On a small-scale, methane production might create a more hospitable atmosphere on the surface of Mars. Additionally, the combustible nature of methane, utilized on Earth as a biogas, could someday provide rocket fuel for spacecraft.

See also Biogeochemical cycles; Chemoautotrophic and chemolithitrophic bacteria; Extremophiles

MICRO ARRAYS • *see* DNA CHIPS AND MICROARRAYS

MICROBIAL FLORA OF THE ORAL CAVITY, DENTAL CARIES

The microbial flora of the oral cavity are rich and extremely diverse. This reflects the abundant nutrients and moisture, and hospitable temperature, and the availability of surfaces on which bacterial populations can develop. The presence of a myriad of **microorganisms** is a natural part of proper oral health. However, an imbalance in the microbial flora can lead to the production of acidic compounds by some microorganisms that can damage the teeth and gums. Damage to the teeth is referred to a dental caries.

Microbes can adhere to surfaces throughout the oral cavity. These include the tongue, epithelial cells lining the roof of the mouth and the cheeks, and the hard enamel of the teeth. In particular, the microbial communities that exist on the surface of the teeth are known as dental **plaque**. The adherent communities also represent a biofilm. Oral biofilms develop over time into exceedingly complex communities. Hundreds of species of **bacteria** have been identified in such biofilms.

Development of the adherent populations of microorganisms in the oral cavity begins with the association and irreversible adhesion of certain bacteria to the tooth surface. Components of the host oral cavity, such as proteins and glycoproteins from the saliva, also adhere. This early coating is referred to as the conditioning film. The conditioning film alters the chemistry of the tooth surface, encouraging the adhesion of other microbial species. Over time, as the biofilm thickens, gradients develop within the biofilm. For example, oxygen may be relatively plentiful at the outer extremity of the biofilm, with the core of the biofilm being essentially oxygen-free. Such environmental alterations promote the development of different types of bacteria in different regions of the biofilm.

This changing pattern represents what is termed bacterial succession. Examples of some bacteria that are typically present as primary colonizers include *Streptococcus*, *Actinomyces*, *Neisseria*, and *Veillonella*. Examples of secondary colonizers include *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Capnocytophaga* species. With further time, another group of bacteria can become associated with the adherent community. Examples of these bacteria include *Campylobacter rectus*, *Eikenella corrodens*, *Actinobacillus actinomycetemcomitans*, and the oral **spirochetes** of the genus *Treponema*.

Under normal circumstances, the microbial flora in the oral cavity reaches equilibrium, where the chemical by-products of growth of some microbes are utilized by other microbes for their growth. Furthermore, the metabolic activities of some bacteria can use up oxygen, creating conditions that are favorable for the growth of those bacteria that require oxygen-free conditions.

This equilibrium can break down. An example is when the diet is high in sugars that can be readily used by bacteria. The **pH** in the adherent community is lowered, which selects for the predominance of acid-loving bacteria, principally *Streptococcus mutans* and *Lactobacillus* species. These species can produce acidic products. The resulting condition is termed dental caries. Dental caries is the second most common of all maladies in humans, next only to the common **cold**. It is the most important cause of tooth loss in people under 10 years of age.

Dental caries typically proceeds in stages. Discoloration and loosening of the hard enamel covering of the tooth precedes the formation of a microscopic hole in the enamel. The hole subsequently widens and damage to the interior of the tooth usually results. If damage occurs to the core of the tooth, a region containing what is termed pulp, and the roots anchoring the tooth to the jaw, the tooth is usually beyond saving. Removal of the tooth is necessary to prevent accumulation of bacterial products that could pose further adverse health effects.

Dental caries can be lessened or even prevented by coating the surface of the tooth with a protective sealant. This is usually done as soon as a child acquires the second set of teeth. Another strategy to thwart the development of dental caries is the inclusion of a chemical called fluoride in drinking water. Evidence supports the use of fluoride to lessen the predominance of acid-producing bacteria in the oral cavity. Finally, good oral **hygiene** is of paramount importance in den-

tal heath. Regular brushing of the teeth and the avoidance of excessive quantities of sugary foods are very prudent steps to maintaining the beneficial equilibrium microbial equilibrium in the oral cavity.

See also Bacteria and bacterial infection

MICROBIAL FLORA OF THE SKIN

The skin is the primary external coating of the human body. In adults, skin occupies approximately 2.4 square yards (approximately two square meters). Because it is exposed to the environment, the skin is inhabited by a number of **bacteria**. Over much of the body there are hundreds of bacteria per square inch of skin. In more moisture-laden regions, such as the armpit, groin, and in between the toes, bacteria can number upwards of one hundred thousand per square inch.

The majority of the skin microbes are found in the first few layers of the epidermis (the outermost layer of skin) and in the upper regions of the hair follicles. The bacteria found here are mostly *Staphylococcus epidermidis* and species of Corynebacteria, Micrococcus, Mycobacterium, and Pityrosporum. These species are described as being commensal; that is, the association is beneficial for one organism (in this case the microbe) and not harmful to the other organism (the human). They are part of the natural environment of the skin and as such are generally benign.

The skin microflora can also be a protective mechanism. By colonizing the skin, the commensal microbes can restrict the colonization by other, hostile **microorganisms**. This phenomenon is referred to as competitive exclusion. The environment of the skin also predisposes the skin to selective colonization. Glands of the skin secrete compounds called fatty acids. Many organisms will not tolerate these fatty acids. But, the normal microflora of the skin is able to tolerate and grow in the presence of the fatty acids. As well, sweat contains a natural antibiotic known as dermicidin. The normal flora seems to be more tolerant to dermicidin than are invading microbes. Thus, their presence of a normal population of microorganisms on the skin is encouraged by the normal physiological conditions of the body.

Newborn babies do not have established skin microorganisms. Colonization occurs within hours of birth, especially following contact with parents and siblings. The resulting competitive exclusion of more hostile microbes is especially important in the newborn, whose **immune system** is not yet fully developed.

In contrast to the protection they bestow, skin microorganisms can cause infections if they gain entry to other parts of the body, such as through cut or during a surgical procedure, or because of a malfunctioning immune system. Bacteria and other microbes that are normal residents of the skin cause some six to ten percent of common hospital-acquired infections. For example, the **yeast** *Candida albicans* can cause a urinary tract infection. In another example, if the sweat glands malfunction, the resident *Proprionibacterium acnes* can be encouraged to undergo explosive growth. The resulting blockage of the sweat glands and **inflammation** can produce skin irritation and sores. As a final example, the Corynebacterium can cause infection of wounds and heart valve infections if they gain entry to deeper regions of the body.

Other microorganisms that are transient members of the skin population can be a problem. ***Escherichia coli***, normally a resident of the intestinal tract, can be acquired due to poor personal **hygiene**. Another bacterial species, *Staphylococcus aureus*, can be picked up from infected patients in a hospital setting. One on the skin, these disease-causing bacteria can be passed on by touch to someone else directly or to a surface. Fortunately, these problematic bacteria can be easily removed by normal handwashing with ordinary soap. Unfortunately, this routine procedure is sometimes not as widely practiced as it should be. Organizations such as the American Society for Microbiology have mounted campaigns to increase awareness of the benefits of hand washing.

However, handwashing is not totally benign. Particularly harsh soaps, or very frequent hand washing (for example, 20–30 times a day) can increase the acidity of the skin, which can counteract some of the protective fatty acid secretions. Also the physical act of washing will shed skin cells. If washing is excessive, the protective microflora will be removed, leaving the newly exposed skin susceptible to colonization by another, potentially harmful microorganism. Health care workers, who scrub their hands frequently, are prone to **skin infections** and damage.

See also Acne, microbial basis of; Bacterial growth and division; Colony and colony formation; Fatty acids; structures and functions; Infection and resistance; Infection control; Microbial flora of the oral cavity, dental caries; Microbial flora of the stomach and gastrointestinal tract

MICROBIAL FLORA OF THE STOMACH AND GASTROINTESTINAL TRACT

The stomach and gastrointestinal tract are not sterile and are colonized by **microorganisms** that perform functions beneficial to the host, including the manufacture of essential vitamins, and the prevention of colonization by undesirable microbes.

The benefits of the close relationship between the microorganisms and the host also extends to the microbes. Microorganisms are provided with a protected place to live and their environment—rich in nutrients—and is relatively free from predators.

This mutually beneficial association is always present. At human birth, the stomach and gastrointestinal tract are usually sterile. But, with the first intake of food, colonization by **bacteria** commences. For example, in breast-fed babies, most of the intestinal flora consists of bacteria known as bifidobacteria. As breast milk gives way to bottled milk, the intestinal flora changes to include enteric bacteria, bacteroides, enterococci, lactobacilli, and clostridia.

The flora of the gastrointestinal tract in animals has been studied intensively. These studies have demonstrated that bacteria are the most numerous microbes present in the stomach and gastrointestinal tract. The composition of the bacterial populations varies from animal to animal, even within a species. Sometimes the diet of an animal can select for the dominance of one or a few bacteria over other species. The situation is similar in humans. Other factors that influence the bacterial make up of the human stomach and gastrointestinal tract include age, cultural conditions, and the use of **antibiotics**. In particular, the use of antibiotics can greatly change the composition of the gastrointestinal flora.

Despite the variation in bacterial flora, the following bacteria tend to be present in the gastrointestinal tract of humans and many animals: ***Escherichia coli***, *Clostridium perfringens*, Enterococci, Lactobacilli, and *Bacteroides*.

The esophagus is considered to be part of the gastrointestinal tract. In this region, the bacteria present are usually those that have been swallowed with the food. These bacteria do not normally survive the journey through the highly acidic stomach. Only bacteria that can tolerate strongly acidic environments are able to survive in the stomach. One bacterium that has been shown to be present in the stomach of many people is *Helicobacter pylori*. This bacterium is now known to be the leading cause of stomach ulcers. In addition, very convincing evidence is mounting that links the bacterium to the development of stomach and intestinal cancers.

In humans, the small intestine contains low numbers of bacteria, some 100,000 to 10 million bacteria per milliliter of fluid. To put these numbers into perspective, a laboratory liquid **culture** that has attained maximum bacterial numbers will contain 100 million to one billion bacteria per milliliter. The bacterial flora of this region consists mostly of lactobacilli and *Enterococcus faecalis*. The lower regions of the small intestine contain more bacteria and a wider variety of species, including coliform bacteria such as *Escherichia coli*.

In the large intestine, the bacterial numbers can reach 100 billion per milliliter of fluid. The predominant species are anaerobic bacteria, which do not grow in the presence of oxygen. These include anaerobic **lactic acid bacteria**, *Bacteroides*, and *Bifidobacterium bifidum*. The bacteria numbers and composition in the large intestine is effectively that of fecal material.

The massive numbers of bacteria in the large intestine creates a great special variation in the flora. Sampling the intestinal wall at different locations will reveal differences in the species of bacteria present. As well, sampling any given point in the intestine will reveal differences in the bacterial population at various depths in the adherent growth on the intestinal wall.

Some bacteria specifically associate with certain cells in the gastrointestinal tract. Gram-positive bacteria such as **streptococci** and lactobacilli often adhere to cells by means of capsules surrounding the bacteria. Gram-negative bacteria such as *Escherichia coli* can adhere to receptors on the intestinal epithelial cells by means of the bacterial appendage called fimbriae.

The importance of the microbial flora of the stomach and gastrointestinal tract has been demonstrated by comparison of the structure and function of the digestive tracts of normal animals and notobiotic animals. The latter animals lack bacteria. The altered structure of the intestinal tract in the notobiotic animals is less efficient in terms of processing food and absorbing nutrients. Additionally, in animals like cows that consume cellulose, the **fermentation** activity of intestinal microorganisms is vital to digestion. Thus, the flora of the stomach and intestinal tract is very important to the health of animals including humans.

See also Enterobacteriaceae; Probiotics; Salmonella food poisoning

MICROBIAL GENETICS

Microbial genetics is a branch of genetics concerned with the transmission of hereditary characters in **microorganisms**. Within the usual definition, microorganisms include prokaryotes like **bacteria**, unicellular or mycelial **eukaryotes** e.g., yeasts and other **fungi**, and **viruses**, notably bacterial viruses (bacteriophages). Microbial genetics has played a unique role in developing the fields of molecular and cell biology and also has found applications in medicine, agriculture, and the food and pharmaceutical industries.

Because of their relative simplicity, microbes are ideally suited for combined biochemical and genetic studies, and have been successful in providing information on the **genetic code** and the regulation of **gene** activity. The **operon** model formulated by French biologists **François Jacob** (1920–) and **Jacques Monod** (1910–1976) in 1961, is one well known example. Based on studies on the induction of **enzymes** of lactose catabolism in the bacterium *Escherichia coli,* the operon has provided the groundwork for studies on gene expression and regulation, even up to the present day. The many applications of microbial genetics in medicine and the pharmaceutical industry emerge from the fact that microbes are both the causes of disease and the producers of **antibiotics**. Genetic studies have been used to understand variation in pathogenic microbes and also to increase the yield of antibiotics from other microbes.

Hereditary processes in microorganisms are analogous to those in multicellular organisms. In both prokaryotic and eukaryotic microbes, the genetic material is **DNA**; the only known exceptions to this rule are the **RNA** viruses. **Mutations**, heritable changes in the DNA, occur spontaneously and the rate of mutation can be increased by mutagenic agents. In practice, the susceptibility of bacteria to mutagenic agents has been used to identify potentially hazardous chemicals in the environment. For example, the Ames test was developed to evaluate the mutagenicity of a chemical in the following way. Plates containing a medium lacking in, for example, the nutrient histidine are inolculated with a histidine requiring strain of the bacterium *Salmonella typhimurium*. Thus, only cells that revert back to the wild type can grow on the medium. If plates are exposed to a mutagenic agent, the increase in the number of **mutants** compared with unexposed plates can be observed and a large number of revertants would indicate a strong muta-

genic agent. For such studies, microorganisms offer the advantage that they have short mean generation times, are easily cultured in a small space under controlled conditions and have a relatively uncomplicated structure.

Microorganisms, and particularly bacteria, were generally ignored by the early geneticists because of their small in size and apparent lack of easily identifiable variable traits. Therefore, a method of identifying variation and mutation in microbes was fundamental for progress in microbial genetics. As many of the mutations manifest themselves as metabolic abnormalities, methods were developed by which microbial mutants could be detected by selecting or testing for altered phenotypes. Positive **selection** is defined as the detection of mutant cells and the rejection of unmutated cells. An example of this is the selection of **penicillin** resistant mutants, achieved by growing organisms in media containing penicillin such that only resistant colonies grow. In contrast, negative selection detects cells that cannot perform a certain function and is used to select mutants that require one or more extra growth factors. Replica plating is used for negative selection and involves two identical prints of **colony** distributions being made on plates with and without the required nutrients. Those microbes that do not grow on the plate lacking the nutrient can then be selected from the identical plate, which does contain the nutrient.

The first attempts to use microbes for genetic studies were made in the USA shortly before World War II, when George W. Beadle (1903–1989) and **Edward L. Tatum** (1909–1975) employed the fungus, *Neurospora,* to investigate the genetics of tryptophan **metabolism** and nicotinic acid synthesis. This work led to the development of the "one gene one enzyme" hypothesis. Work with bacterial genetics, however, was not really begun until the late 1940s. For a long time, bacteria were thought to lack sexual reproduction, which was believed to be necessary for mixing genes from different individual organisms—a process fundamental for useful genetic studies. However, in 1947, **Joshua Lederberg** (1925–) working with Edward Tatum demonstrated the exchange of genetic factors in the bacterium, *Escherichia coli.* This process of DNA transfer was termed **conjugation** and requires cell-to-cell contact between two bacteria. It is controlled by genes carried by **plasmids**, such as the fertility (F) factor, and typically involves the transfer of the plasmid from donor torecipient cell. Other genetic elements, however, including the donor cell chromosome, can sometimes also be mobilized and transferred. Transfer to the host chromosome is rarely complete, but can be used to map the order of genes on a bacterial genome.

Other means by which foreign genes can enter a bacterial cell include **transformation**, transfection, and **transduction**. Of the three processes, transformation is probably the most significant. Evidence of transformation in bacteria was first obtained by the British scientist, Fred Griffith (1881–1941) in the late 1920s working with *Streptococcus pneumoniae* and the process was later explained in the 1930s by **Oswald Avery** (1877–1955) and his associates at the Rockefeller Institute in New York. It was discovered that certain bacteria exhibit competence, a state in which cells are able to take up free DNA released by other bacteria. This is the process known as transformation, however, relatively few microorganisms can be naturally transformed. Certain laboratory procedures were later developed that make it possible to introduce DNA into bacteria, for example electroporation, which modifies the bacterial membrane by treatment with an electric field to facilitate DNA uptake. The latter two processes, transfection and transduction, involve the participation of viruses for nucleic acid transfer. Transfection occurs when bacteria are transformed with DNA extracted from a bacterial virus rather than from another bacterium. Transduction involves the transfer of host genes from one bacterium to another by means of viruses. In generalized transduction, defective virus particles randomly incorporate fragments of the cell DNA; virtually any gene of the donor can be transferred, although the efficiency is low. In specialized transduction, the DNA of a temperate virus excises incorrectly and brings adjacent host genes along with it. Only genes close to the integration point of the virus are transduced, and the efficiency may be high.

After the discovery of DNA transfer in bacteria, bacteria became objects of great interest to geneticists because their rate of reproduction and mutation is higher than in larger organisms; i.e., a mutation occurs in a gene about one time in 10,000,000 gene duplications, and one bacterium may produce 10,000,000,000 offspring in 48 hours. Conjugation, transformation, and transduction have been important methods for mapping the genes on the **chromosomes** of bacteria. These techniques, coupled with restriction enzyme analysis, **cloning** DNA sequencing, have allowed for the detailed studies of the bacterial chromosome. Although there are few rules governing gene location, the genes encoding enzymes for many biochemical pathways are often found tightly linked in operons in prokaryotes. Large scale sequencing projects revealed the complete DNA sequence of the genomes of several prokaryotes, even before eukaryotic genomes were considered.

See also Bacterial growth and division; Bacteriophage and bacteriophage typing; Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Fungal genetics; Mutations and mutagenesis; Viral genetics; Viral vectors in gene therapy

MICROBIAL SYMBIOSIS

Symbiosis is generally defined as a condition where two dissimilar organisms live together in an intimate associate that sees both organisms benefit. Microbial symbiosis tends to be bit broader in definition, being defined as the co-existence of two **microorganisms**.

Microbial symbiosis can be evident as several different patterns of co-existence. One pattern is known as mutualism. In this relationship, both organisms benefit. Another type of relationship is called commensalism. Here the relationship is beneficial to one of the organisms and does no harm to the other.

Another relationship known as parasitism produces a benefit to one organism at the expense of the other organism. Parasitism is not considered to be a symbiosis between a microorganism and the host.

Microbial symbiosis has been a survival feature of **bacteria** since their origin. The best example of this is the presence of the energy factories known as mitochondria in eukaryotic cells. Mitochondria arose because of the symbiosis between an ancient bacterium and a eukaryote. Over evolutionary time the symbiosis became permanent, and the bacterium became part of the host. However, even to the present day the differences in constitution and arrangement of the genetic material of mitochondria and the host cell's **nucleus** attests to the symbiotic origin of mitochondria.

There are several well-known examples of bacterial mutualism. The first example is the presence of huge numbers of bacteria in the intestinal tract of warm-blooded animals such as humans. Fully 10 percent of the dry weight of a human consists of bacteria. The bacteria act to break down foodstuffs, and so directly participate in the digestive process. As well, some of the intestinal bacteria produce products that are crucial to the health of the host. For example. In humans, some of the gut bacteria manufacture vitamin K, vitamin B_{12}, biotin, and riboflavin. These vitamins are important to the host but are not made by the host. The bacteria benefit by inhabiting an extremely hospitable environment. The natural activities and numbers of the bacteria also serve to protect the host from colonization by disease-causing microorganisms. The importance of this type of symbiosis is exemplified by the adverse health effects to the host that can occur when the symbiotic balance is disturbed by antibiotic therapy.

A second example of symbiotic mutualism is the colonization of the nodules of leguminous plants by bacteria of the genus *Rhizobium*. The bacteria convert free nitrogen gas into a form of nitrogen called nitrate. This form of nitrogen can be readily utilized by the plant, which cannot otherwise use the gaseous form of nitrogen. The plant benefits by acquiring a readily available nitrogen source, and, as for the intestinal bacteria, *Rhizobium* benefits by virtue of the hospitable environment for growth.

The skin is colonized by a number of different types of bacteria, such as those from the genera *Staphylococcus* and *Streptococcus*. The bacteria are exposed to a read supply of nutrients, and their colonization of the skin helps protect that surface from colonization by less desirable microorganisms.

Microbial symbiosis can be exquisite. An example is the Gram-negative bacterium *Xenorhabdus nematophilus*. This bacterium lives in a nematode called *Steinernema carpocapsae*. Both organisms require the other for their survival. Thus the symbiosis is obligatory. The bacterium in fact supplies toxins that are used to kill insect that the nematode infects.

The scope of microbial symbiosis in nature is vast. In the 1970s the existence of thermal vents on the ocean floor was discovered. It has since been shown that the basis of the lush ecosystem surrounding these sources of heat is bacteria, and that a significant proportion of these bacteria live in symbiosis with the tubular worm-like creatures that thrives in this environment. In fact, the bacteria are absolutely required for the utilization of nutrients by the tube worms.

Numerous other examples of microbial symbiosis exist in nature. Animals, plants as exotic as coral, insects, fish, and birds all harbor microorganisms that assist them in their survival. Indeed, the ancient roots of microbial symbiosis may be indicative of a more cooperative **evolution** of life on Earth than prior studies indicated.

See also Bacterial kingdoms; Microbial taxonomy

MICROBIAL TAXONOMY

Microbial taxonomy is a means by which **microorganisms** can be grouped together. Organisms having similarities with respect to the criteria used are in the same group, and are separated from the other groups of microorganisms that have different characteristics.

There are a number of taxonomic criteria that can be used. For example, numerical taxonomy differentiates microorganisms, typically **bacteria**, on their phenotypic characteristics. Phenotypes are the appearance of the microbes or the manifestation of the genetic character of the microbes. Examples of phenotypic characteristics include the Gram stain reaction, shape of the bacterium, size of the bacterium, where or not the bacterium can propel itself along, the capability of the microbes to grow in the presence or absence of oxygen, types of nutrients used, chemistry of the surface of the bacterium, and the reaction of the **immune system** to the bacterium.

Numerical taxonomy typically invokes a number of these criteria at once. The reason for this is that if only one criterion was invoked at a time there would be a huge number of taxonomic groups, each consisting of only one of a few microorganisms. The purpose of grouping would be lost. By invoking several criteria at a time, fewer groups consisting of larger number of microorganisms result.

The groupings result from the similarities of the members with respect to the various criteria. A so-called similarity coefficient can be calculated. At some imposed threshold value, microorganisms are placed in the same group.

A well-known example of taxonomic characterization is the kingdom, division, class, family, genus, species and strain divisions. Such a "classical" bacterial organization, which is typified by the Bergey's Manual of Determinative Bacteriology, is based on metabolic, immunological, and structural characteristics. Strains, for example, are all descended from the same organism, but differ in an aspect such as the antigenic character of a surface molecule.

Microbial taxonomy can create much order from the plethora of microorganisms. For example, the **American Type Culture Collection** maintains the following, which are based on taxonomic characterization (the numbers in brackets indicate the number of individual organisms in the particular category): algae (120), bacteria (14400), **fungi** (20200), **yeast** (4300), **protozoa** (1090), animal **viruses** (1350), **plant viruses** (590), and bacterial viruses (400). The actual number of microorganisms in each category will continue to change as new microbes are isolated and classified. The general structure, however, of this classical, so-called phenetic system will remain the same.

The separation of the microorganisms is typically represented by what is known as a dendrogram. Essentially, a den-

drogram appears as a tree oriented on a horizontal axis. The dendrogram becomes increasingly specialized—that is, the similarity coefficient increases—as the dendrogram moves from the left to the right. The right hand side consists of the branches of the trees. Each branch contains a group of microorganisms.

The dendrogram depiction of relationships can also be used for another type of microbial taxonomy. In this second type of taxonomy, the criterion used is the shared evolutionary heritage. This heritage can be determined at the genetic level. This is termed molecular taxonomy.

Molecular microbial taxonomy relies upon the generation and inheritance of genetic **mutations** that is the replacement of a nucleotide building block of a **gene** by another nucleotide. Sometimes the mutation confers no advantage to the microorganism and so is not maintained in subsequent generations. Sometimes the mutation has an adverse effect, and so is actively suppressed or changed. But sometimes the mutation is advantageous for the microorganism. Such a mutation will be maintained in succeeding generations.

Because mutations occur randomly, the divergence of two initially genetically similar microorganisms will occur slowly over evolutionary time (millions of years). By sequencing a target region of genetic material, the relatedness or dissimilarity of microorganisms can be determined. When enough microorganisms have been sequenced, relationships can be established and a dendrogram constructed.

For a meaningful genetic categorization, the target of the comparative sequencing must be carefully chosen. Molecular microbial taxonomy of bacteria relies on the sequence of **ribonucleic acid** (**RNA**), dubbed 16S RNA, that is present in a subunit of prokaryotic **ribosomes**. Ribosomes are complexes that are involved in the manufacture of proteins using messenger RNA as the blueprint. Given the vital function of the 16S RNA, any mutation tends to have a meaningful, often deleterious, effect on the functioning of the RNA. Hence, the **evolution** (or change) in the 16S RNA has been very slow, making it a good molecule with which to compar microorganisms that are billions of years old.

Molecular microbial taxonomy has been possible because of the development of the technique of the **polymerase chain reaction**. In this technique a small amount of genetic material can be amplified to detectable quantities

The use of the chain reaction has produced a so-called bacterial phylogenetic tree. The structure of the tree is even now evolving. But the current view has the tree consisting of three main branches. One branch consists of the bacteria. There are some 11 distinct groups within the bacterial branch. Three examples are the green non-sulfur bacteria, Gram- positive bacteria, and cyanobacteria.

The second branch of the evolutionary tree consists of the Archae, which are thought to have been very ancient bacteria that diverged from both bacteria and eukaryotic organisms billions of years ago. Evidence to date places the Archae a bit closer on the tree to bacteria than to the final branch (the Eucarya). There are three main groups in the archae: halophiles (salt-loving), methanogens, and the extreme thermophiles (heat loving).

Finally, the third branch consists of the Eucarya, or the eukaryotic organisms. Eucarya includes organisms as diverse as fungi, plants, **slime molds** and animals (including humans).

See also Bacterial kingdoms; Genetic identification of microorganisms

MICROBIOLOGY, CLINICAL

Clinical microbiology is concerned with infectious **microorganisms**. Various **bacteria**, algae and **fungi** are capable of causing disease.

Disease causing microorganisms have been present for millennia. Examples include **anthrax**, **smallpox**, bacterial **tuberculosis**, plague, **diphtheria**, **typhoid fever**, bacterial diarrhea, and **pneumonia**. While modern technological advances, such as mass **vaccination**, have reduced the threat of some of these diseases, others remain a problem. Some illnesses are re-emerging, due to acquisition of resistance to many **antibiotics**. Finally, other diseases, such as the often lethal hemorrhagic fever caused by the **Ebola virus**, have only been recognized within the past few decades.

Many bacterial diseases have only been recognized since the 1970s. These include **Legionnaires' disease**, Campylobacter infection of poultry, **toxic shock syndrome**, hemolytic uremic syndrome, **Lyme disease**, peptic ulcer disease, human ehrlichiosis, and a new strain of cholera. Clinical microbiology research and techniques were vital in identifying the cause of these maladies, and in seeking treatments and ultimately a cure for each malady.

Clinical microbiology involves both the detection and identification of disease-causing microorganisms, and research to find more effective means of treating the infection or preventing infections from occurring. The symptoms of the ailment, and the shape, **Gram stain** reaction (in the case of bacteria), and biochemical reactions of an unknown organism are used to diagnose the cause of an infection. Knowledge of the identity of the microbe suggests means of treatment, such as the application of antibiotics. Many clinical microbiologists are also researchers. In many cases, the molecular basis of an organism's disease-causing capability is not clear. Unraveling the reasons why a disease is produced can help find ways to prevent the disease.

There are several groups or categories of bacteria that are of medical importance. They are grouped into five categories based on their shape and reaction to the Gram stain. These criteria apply to the light **microscope**, as typically a first step in the identification of bacteria in an infection is the light microscope examination of material obtained from the infection or from a **culture**. The groups are Gram-positive bacilli (rod-shaped bacteria), Gram negative bacilli, Gram positive cocci (round bacteria), Gram negative cocci, and bacteria that react atypically to the Gram stain.

A group of spiral shaped bacteria called **spirochetes** are responsible for leptospirosis in dogs, and **syphilis** and Lyme disease in humans. These bacteria are easily identified under the light microscope because of their wavy shape and

Laboratory technicians.

Laboratory technicians.

corkscrew movement (courtesy of rigid internal filaments that run the length of the bacterium). A related group (a genus) of spiral shaped bacteria is *Spirilla.* These bacteria move by means of external flagella, not by means of the internal filaments. Two members of *Spirilla* are important disease-causing bacteria. The first is *Campylobacter jejuni*, which frequently contaminates raw meat such as poultry and drinking water, and which is the cause of diarrhea, especially in children. The second bacterial type is *Helicobacter pylori*, which grows in the stomach and has been demonstrated to be the principle cause of stomach ulcers.

Another group of clinically relevant bacteria is termed pseudomonads. This group contains many different types of bacteria. They all are similar in shape and biochemical behavior to a species called *Pseudomonas aeruginosa*. Most pseudomonads, like *Pseudomonas aeruginosa* live in water and the soil. They cause a variety of ailments. *Bordetella pertussis* causes whooping cough, *Legionella pneumophila* causes Legionnaires' disease, *Neisseria gonorrhoeae* causes **gonorrhea**, and *Neisseria meningitides* causes bacterial **meningitis**. *Pseudomonas aeruginosa* is the quintessential so-called opportunitistic pathogen; a bacteria that does not normally cause an infection but can do so in a compromised host. Examples of such infections are the chronic lung infections in those who have certain forms of cystic fibrosis, and infections in burn victims.

Yet another group of bacteria of medical importance live in the intestinal tracts of humans, other mammals and even in birds and reptiles. These are the enteric bacteria. The best-known enteric bacteria is ***Escherichia coli***, the cause of intestinal illness and sometimes even more severe damage to the urinary tract and kidneys from ingestion of contaminated water or food ("hamburger disease"). Other noteworthy enteric bacteria are *Shigella dysenteriae* (**dysentery**), *Salmonella* species **gastroenteritis** and typhoid fever), *Yersinia pestis* (**bubonic plague**), and *Vibrio cholerae* (cholera).

Bacteria including *Staphylococcus* and *Streptococcus,* which normally live on the skin, can cause infection when they gain entry to other pasts of the body. The illnesses they cause (such as **strep throat**, pneumonia, and blood infection, as examples), and the number of cases of these illnesses, make them the most clinically important disease-causing bacteria known to man. *Staphylococcus aureus* is the leading cause of hospital acquired infections of all the gram-positive bacteria. Ominously, a strain of this organism now exists that is resist-

ant to many antibiotics. As this strain increases its worldwide distribution, *Staphylococcus* infections will become an increasing problem.

Bacteria that normally live in the mouth are responsible for the formation of dental **plaque** on the surface of teeth. Protected within the plaque, the bacteria produce acid that eats away tooth enamel, leading to the development of a cavity.

A few examples of other clinically important bacteria are *Bacillus anthracis* (anthrax), *Clostridium tetani* (**tetanus**), *Mycobacterium tuberculosis* (tuberculosis), *Corynebacterium diphtheriae* (diphtheria), various Rickettsias (Rocky Mountain Spotted Fever, **Q fever**), *Chlamydia trachomatis* (chlamydia).

Fungi and **yeast** are also capable of causing infection. For example, the fungal genus *Tinea* comprises species that cause conditions commonly described as "jock itch" and "athlete's foot." Scalp infections are also caused by some species of fungus.

Viruses are also the cause of a variety of infections. **Inflammation** of the coating of nerve cells (meningitis) and brain tissue (encephalitis), and infections of tissues in the mouth, bronchial tract, lungs and intestinal tract result from infection by various viruses.

See also Blood borne infections; Cold, viruses; Laboratory techniques in microbiology; Viruses and response to viral infection; Yeast, infectious

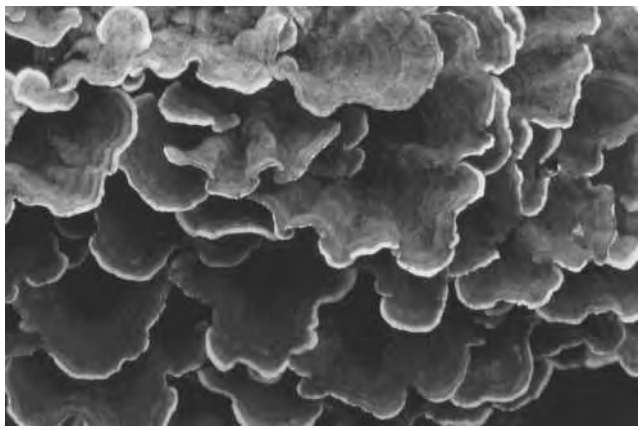
MICROBIOLOGY, HISTORY OF • *see* HISTORY OF MICROBIOLOGY

MICROORGANISMS

Microorganisms are minute organisms of microscopic dimensions, too small to be seen by the eye alone. To be viewed, microorganisms must be magnified by an optical or **electron microscope**. The most common types of microorganisms are **viruses**, **bacteria**, blue-green bacteria, some algae, some **fungi**, yeasts, and protozoans.

Viruses, bacteria, and blue-green bacteria are all prokaryotes, meaning that they do not have an organized cell **nucleus** separated from the protoplasm by a membrane-like envelope. Viruses are the simplest of the prokaryotic life forms. They are little more than simple genetic material, either **DNA** (**deoxyribonucleic acid**) or **RNA** (**ribonucleic acid**), plus associated proteins of the viral shell (called a capsid) that together comprise an infectious agent of cells. Viruses are not capable of independent reproduction. They reproduce by penetrating a host cell and diverting much of its metabolic and reproductive physiology to the reproduction of copies of the virus.

The largest kingdom of prokaryotes is the Monera. In this group, the genetic material is organized as a single strand of DNA, neither meiosis nor mitosis occurs, and reproduction is by asexual cellular division. Bacteria (a major division of the Monera) are characterized by rigid or semi-rigid cell walls, propagation by binary division of the cell, and a lack of mitosis. Blue-green bacteria or cyanobacteria (also in the Monera) use **chlorophyll** dispersed within the **cytoplasm** as the primary light-capturing pigment for their **photosynthesis**.



A lichen growing on wood.

Many microorganisms are eukaryotic organisms, having their nuclear material organized within a nucleus bound by an envelope. **Eukaryotes** also have paired **chromosomes** of DNA, which can be seen microscopically during mitosis and meiosis. They also have a number of other discrete cellular organelles.

Protists are a major kingdom of eukaryotes that includes microscopic protozoans, some fungi, and some algae. Protists have flagellated spores, and mitochondria and plastids are often, but not always, present. Protozoans are single-celled microorganisms that reproduce by binary fission and are often motile, usually using cilia or flagellae for propulsion; some protozoans are colonial.

Fungi are heterotrophic organisms with chitinous cell walls, and they lack flagella. Some fungi are unicellular microorganisms, but others are larger and have thread-like **hyphae** that form a more complex **mycelium**, which take the form of mushrooms in the most highly developed species. Yeasts are a group of single-celled fungi that reproduce by budding or by cellular fission.

Algae are photosynthetic, non-vascular organisms, many of which are unicellular, or are found in colonies of several cells; these kinds of algae are microscopic.

In summary, microorganisms comprise a wide range of diverse but unrelated groups of tiny organisms, characterized only by their size. As a group, microorganisms are extremely important ecologically as primary producers, and as agents of decay of dead organisms and recycling of the nutrients contained in their biomass. Some species of microorganisms are also important as **parasites** and as other disease-causing agents in humans and other organisms.

See also Bacteria and bacterial infection; Genetic identification of microorganisms; Viruses and responses to viral infection; Microbial flora of the skin; Microbial genetics; Microbial symbiosis; Microbial taxonomy; Microscope and microscopy

MICROSCOPE AND MICROSCOPY

Microscopy is the science of producing and observing images of objects that cannot be seen by the unaided eye. A microscope is an instrument that produces the image. The primary function of a microscope is to resolve, that is distinguish, two closely spaced objects as separate. The secondary function of a microscope is to magnify. Microscopy has developed into an exciting field with numerous applications in biology, geology, chemistry, physics, and technology.

Since the time of the Romans, it was realized that certain shapes of glass had properties that could magnify objects. By the year 1300, these early crude lenses were being used as corrective eyeglasses. It wasn't until the late 1500s, however, that the first compound microscopes were developed.

Robert Hooke (1635–1703) was the first to publish results on the microscopy of plants and animals. Using a simple two-lens compound microscope, he was able to discern the cells in a thin section of cork. The most famous microbiologist was Antoni van Leeuwenhoek (1632–1723) who, using just a single lens microscope, was able to describe organisms and tissues, such as **bacteria** and red blood cells, which were previously not known to exist. In his lifetime, Leeuwenhoek built over 400 microscopes, each one specifically designed for one specimen only. The highest resolution he was able to achieve was about 2 micrometers.

By the mid-nineteenth century, significant improvements had been made in the light microscope design, mainly due to refinements in lens grinding techniques. However, most of these lens refinements were the result of trial and error rather than inspired through principles of physics. Ernst Abbé (1840–1905) was the first to apply physical principles to lens design. Combining glasses with different refracting powers into a single lens, he was able to reduce image distortion significantly. Despite these improvements, the ultimate resolution of the light microscope was still limited by the wavelength of light. To resolve finer detail, something with a smaller wavelength than light would have to be used.

In the mid-1920s, Louis de Broglie (1892–1966) suggested that electrons, as well as other particles, should exhibit wave like properties similar to light. Experiments on electron beams a few years later confirmed de Broglie's hypothesis. Electrons behave like waves. Of importance to microscopy was the fact that the wavelength of electrons is typically much smaller than the wavelength of light. Therefore, the limitation imposed on the light microscope of 0.4 micrometers could be significantly reduced by using a beam of electrons to illuminate the specimen. This fact was exploited in the 1930s in the development of the **electron microscope**.

There are two types of electron microscope, the transmission electron microscope (TEM) and the scanning electron microscope (SEM). The TEM transmits electrons through an extremely thin sample. The electrons scatter as they collide with the atoms in the sample and form an image on a photographic film below the sample. This process is similar to a medical x ray, where x rays (very short wavelength light) are transmitted through the body and form an image on photographic film behind the body. By contrast, the SEM reflects a narrow beam of electrons off the surface of a sample and detects the reflected electrons. To image a certain area of the sample, the electron beam is scanned in a back and forth motion parallel to the sample surface, similar to the process of mowing a square section of lawn. The chief differences between the two microscopes are that the TEM gives a two-dimensional picture of the interior of the sample while the SEM gives a three-dimensional picture of the surface of the sample. Images produced by SEM are familiar to the public, as in television commercials showing pollen grains or dust mites.

For the light microscope, light can be focused and bent using the refractive properties of glass lenses. To bend and focus beams of electrons, however, it is necessary to use magnetic fields. The magnetic lens, which focuses the electrons, works through the physical principle that a charged particle, such as an electron that has a negative charge, will experience a force when it is moving in a magnetic field. By positioning magnets properly along the electron beam, it is possible to bend the electrons in such a way as to produce a magnified image on a photographic film or a fluorescent screen. This same principle is used in a television set to focus electrons onto the television screen to give the appropriate images.

Electron microscopes are complex and expensive. To use them effectively requires extensive training. They are rarely found outside the research laboratory. Sample preparation can be extremely time consuming. For the TEM, the sample must be ground extremely thin, less than 0.1 micrometer, so that the electrons will make it through the sample. For the SEM, the sample is usually coated with a thin layer of gold to increase its ability to reflect electrons. Therefore, in electron microscopy, the specimen can't be living. Today, the best TEMs can produce images of the atoms in the interior of a sample. This is a factor of a 1,000 better than the best light microscopes. The SEM, on the other hand, can typically distinguish objects about 100 atoms in size.

In the early 1980s, a new technique in microscopy was developed which did not involve beams of electrons or light to produce an image. Instead, a small metal tip is scanned very close to the surface of a sample and a tiny electric current is measured as the tip passes over the atoms on the surface. The microscope that works in this manner is the scanning tunneling microscope (STM). When a metal tip is brought close to the sample surface, the electrons that surround the atoms on the surface can actually "tunnel through" the air gap and produce a current through the tip. This physical phenomenon is called tunneling and is one of the amazing results of quantum physics. If such phenomenon could occur with large objects, it would be possible for a baseball to tunnel through a brick wall with no damage to either. The current of electrons that tunnel through the air gap is very much dependent on the width of the gap and therefore the current will rise and fall in succession with the atoms on the surface. This current is then amplified and fed into a computer to produce a three dimensional image of the atoms on the surface.

Without the need for complicated magnetic lenses and electron beams, the STM is far less complex than the electron microscope. The tiny tunneling current can be simply amplified through electronic circuitry similar to circuitry that is

Researcher using light microscope to examine cell cultures.

used in other electronic equipment, such as a stereo. In addition, the sample preparation is usually less tedious. Many samples can be imaged in air with essentially no preparation. For more sensitive samples that react with air, imaging is done in vacuum. A requirement for the STM is that the samples be electrically conducting, such as a metal.

There have been numerous variations on the types of microscopy outlined so far. A sampling of these is: acoustic microscopy, which involves the reflection of sound waves off a specimen; x-ray microscopy, which involves the transmission of x rays through the specimen; near field optical microscopy, which involves shining light through a small opening smaller than the wavelength of light; and atomic force microscopy, which is similar to scanning tunneling microscopy but can be applied to materials that are not electrically conducting, such as quartz.

One of the most amazing recent developments in microscopy involves the manipulation of individual atoms. Through a novel application of the STM, scientists at IBM were able to arrange individual atoms on a surface and spell out the letters "IBM." This has opened up new directions in microscopy, where the microscope is both an instrument with which to observe and to interact with microscopic objects. Future trends in microscopy will most likely probe features within the atom.

See also Electron microscope, transmission and scanning; Electron microscopic examination of microorganisms; Laboratory techniques in immunology; Laboratory techniques in microbiology

MILLER-UREY EXPERIMENT

A classic experiment in **molecular biology**, the Miller-Urey experiment, established that the conditions that existed in Earth's primitive atmosphere were sufficient to produce amino acids, the subunits of proteins comprising and required by living organisms. In essence, the Miller-Urey experiment fundamentally established that Earth's primitive atmosphere was capable of producing the building blocks of life from inorganic materials.

In 1953, University of Chicago researchers **Stanley L. Miller** and Harold C. Urey set up an experimental investigation into the molecular origins of life. Their innovative experimental design consisted of the introduction of the molecules thought to exist in early Earth's primitive atmosphere into a closed chamber. Methane (CH_4), hydrogen (H_2), and ammonia (NH_3) gases were introduced into a moist environment above a water-containing flask. To simulate primitive lightning discharges, Miller supplied the system with electrical current.

After a few days, Miller observed that the flask contained organic compounds and that some of these compounds were the amino acids that serve as the essential building blocks of protein. Using chromatological analysis, Miller continued his experimental observations and confirmed the ready formation of amino acids, hydroxy acids, and other organic compounds.

Although the discovery of amino acid formation was of tremendous significance in establishing that the raw materials of proteins were easily to obtain in a primitive Earth environment, there remained a larger question as to the nature of the origin of genetic materials mdash; in particular the origin of **DNA** and **RNA** molecules.

Continuing on the seminal work of Miller and Urey, in the early 1960s Juan Oro discovered that the nucleotide base adenine could also be synthesized under primitive Earth conditions. Oro used a mixture of ammonia and hydrogen cyanide (HCN) in a closed aqueous environment.

Oro's findings of adenine, one of the four nitrogenous bases that combine with a phosphate and a sugar (deoxyribose for DNA and ribose for RNA) to form the nucleotides represented by the **genetic code**: (adenine (A), thymine (T), guanine (G), and cytosine (C). In RNA molecules, the nitrogenous base uracil (U) substitutes for thymine. Adenine is also a fundamental component of adenosine triphosphate (ATP), a molecule important in many genetic and cellular functions.

Subsequent research provided evidence of the formation of the other essential nitrogenous bases needed to construct DNA and RNA.

The Miller-Urey experiment remains the subject of scientific debate. Scientist continue to explore the nature and composition of Earth's primitive atmosphere and thus, continue to debate the relative closeness of the conditions of the Miller-Urey experiment (e.g., whether or not Miller's application of electrical current supplied relatively more electrical energy than did lightning in the primitive atmosphere). Subsequent experiments using alternative stimuli (e.g., ultraviolet light) also confirm the formation of amino acids from the gases present in the Miller-Urey experiment. During the 1970s and 1980s, astrobiologists and astrophyicists, including American physicist Carl Sagan, asserted that ultraviolet light bombarding the primitive atmosphere was far more energetic that even continual lightning discharges. Amino acid formation is greatly enhanced by the presence of an absorber of ultraviolet radiation such as the hydrogen sulfide molecules (H_2S) also thought to exist in the early Earth atmosphere.

Although the establishment of the availability of the fundamental units of DNA, RNA and proteins was a critical component to the investigation of the origin of biological molecules and life on Earth, the simple presence of these molecules is a long step from functioning cells. Scientists and evolutionary biologists propose a number of methods by which these molecules could concentrate into a crude cell surrounded by a primitive membrane.

See also Biochemistry; DNA (Deoxyribonucleic acid); Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Mitochondrial inheritance

MILLER, STANLEY L. (1930-)

American chemist

Stanley Lloyd Miller is most noted for his experiments that attempted to replicate the chemical conditions that may have first given rise to life on Earth. In the early 1950s he demonstrated that amino acids could have been created under primordial conditions. Amino acids are the fundamental units of life; they join together to form proteins, and as they grow more complex they eventually become nucleic acids, which are capable of replicating. Miller has hypothesized that the oceans of primitive Earth were a mass of molecules, a prebiological "soup," which over the course of a billion years became a living system.

Miller was born in Oakland, California, the younger of two children. His father, Nathan Harry Miller, was an attorney and his mother, Edith Levy Miller, was a homemaker. Miller attended the University of California at Berkeley and received his B.S. degree in 1951. He began his graduate studies at the University of Chicago in 1951.

In an autobiographical sketch entitled "The First Laboratory Synthesis of Organic Compounds under Primitive Earth Conditions," Miller recalled the events that led to his famous experiment. Soon after arriving at the University of Chicago, he attended a seminar given by **Harold Urey** on the origin of the solar system. Urey postulated that the earth was reducing when it was first formed—in other words, there was an excess of molecular hydrogen. Strong mixtures of methane and ammonia were also present, and the conditions in the atmosphere favored the synthesis of organic compounds. Miller wrote that when he heard Urey's explanation, he knew it made sense: "For the nonchemist the justification for this might be explained as follows: it is easier to synthesize an organic compound of biological interest from the reducing atmosphere constituents because less chemical bonds need to be broken and put together than is the case with the constituents of an oxidizing atmosphere."

After abandoning a different project for his doctoral thesis, Miller told Urey that he was willing to design an experiment to test his hypothesis. However, Urey expressed reluctance at the idea because he considered it too time consuming and risky for a doctoral candidate. But Miller persisted, and Urey gave him a year to get results; if he failed he would have to choose another thesis topic. With this strict deadline Miller set to work on his attempt to synthesize organic compounds under conditions simulating those of primitive earth.

Miller and Urey decided that ultraviolet light and electrical discharges would have been the most available sources of energy on Earth billions of years ago. Having done some reading into amino acids, Miller hypothesized that if he applied an electrical discharge to his primordial environment, he would probably get a deposit of hydrocarbons, organic compounds containing carbon and hydrogen. As he remembered in "The First Laboratory Synthesis of Organic Compounds": "We decided that amino acids were the best group of compounds to look for first, since they were the building blocks of proteins and since the analytical methods were at that time relatively well developed." Miller designed an apparatus in which he could simulate the conditions of prebiotic Earth and then measure what happened. A glass unit was made to represent a model ocean, atmosphere, and rain. For the first experiment, he filled the unit with the requisite "primitive atmosphere"—methane, hydrogen, water, and ammonia—and then submitted the mixture to a low-voltage spark over night. There was a layer of hydrocarbons the next morning, but no amino acids.

Miller then repeated the experiment with a spark at a higher voltage for a period of two days. This time he found no visible hydrocarbons, but his examination indicated that glycine, an amino acid, was present. Next, he let the spark run for a week and found what looked to him like seven spots. Three of these spots were easily identified as glycine, alpha-alanine, and beta-alanine. Two more corresponded to a-amino-n-butyric acid and aspartic acid, and the remaining pair he labeled A and B.

At Urey's suggestion, Miller published "A Production of Amino Acids under Possible Primitive Earth Conditions" in May of 1953 after only three-and-a-half months of research. Reactions to Miller's work were quick and startling. Articles evaluating his experiment appeared in major newspapers; when a Gallup poll asked people whether they thought it was possible to create life in a test tube; seventy-nine percent of the respondents said no.

After Miller finished his experiments at the University of Chicago, he continued his research as an F. B. Jewett Fellow at the California Institute of Technology from 1954 to 1955. Miller established the accuracy of his findings by performing further tests to identify specific amino acids. He also ruled out the possibility that **bacteria** might have produced the spots by heating the apparatus in an autoclave for eighteen hours (fifteen minutes is usually long enough to kill any bacteria). Subsequent tests conclusively identified four spots that had previously puzzled him. Although he correctly identified the a-amino-n-butyric acid, what he had thought was aspartic acid (commonly found in plants) was really iminodiacetic acid. Furthermore, the compound he had called A turned out to be sarcosine (N-methyl glycine), and compound B was N-methyl alanine. Other amino acids were present but not in quantities large enough to be evaluated.

Although other scientists repeated Miller's experiment, one major question remained: was Miller's apparatus a true representation of the primitive atmosphere? This question was finally answered by a study conducted on a meteorite that landed in Murchison, Australia, in September 1969. The amino acids found in the meteorite were analyzed and the data compared to Miller's findings. Most of the amino acids Miller had found were also found in the meteorite. On the state of scientific knowledge about the origins of human life, Miller wrote in "The First Laboratory Synthesis of Organic Compounds" that "the synthesis of organic compounds under primitive earth conditions is not, of course, the synthesis of a living organism. We are just beginning to understand how the simple organic compounds were converted to polymers on the primitive earth...nevertheless we are confident that the basic process is correct."

Miller's later research has continued to build on his famous experiment. He is looking for precursors to **ribonucleic acid** (**RNA**). "It is a problem not much discussed because there is nothing to get your hands on," he told Marianne P. Fedunkiw in an interview. He is also examining the natural occurrence of clathrate hydrates, compounds of ice and gases that form under high pressures, on the earth and other parts of the solar system.

Miller has spent most of his career in California. After finishing his doctoral work in Chicago, he spent five years in the department of **biochemistry** at the College of Physicians and Surgeons at Columbia University. He then returned to California as an assistant professor in 1960 at the University of California, San Diego. He became an associate professor in 1962 and eventually full professor in the department of chemistry.

Miller served as president of the International Society for the Study of the Origin of Life (ISSOL) from 1986 to 1989. The organization awarded him the Oparin Medal in 1983 for his work in the field. Outside of the United States, he was recognized as an Honorary Councilor of the Higher Council for Scientific Research of Spain in 1973. In addition, Miller was elected to the National Academy of Sciences. Among Miller's other memberships are the American Chemical Society, the American Association for the Advancement of Science, and the American Society of Biological Chemists.

See also Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Miller-Urey experiment

MILSTEIN, CÉSAR (1927-2002)

Argentine English biochemist

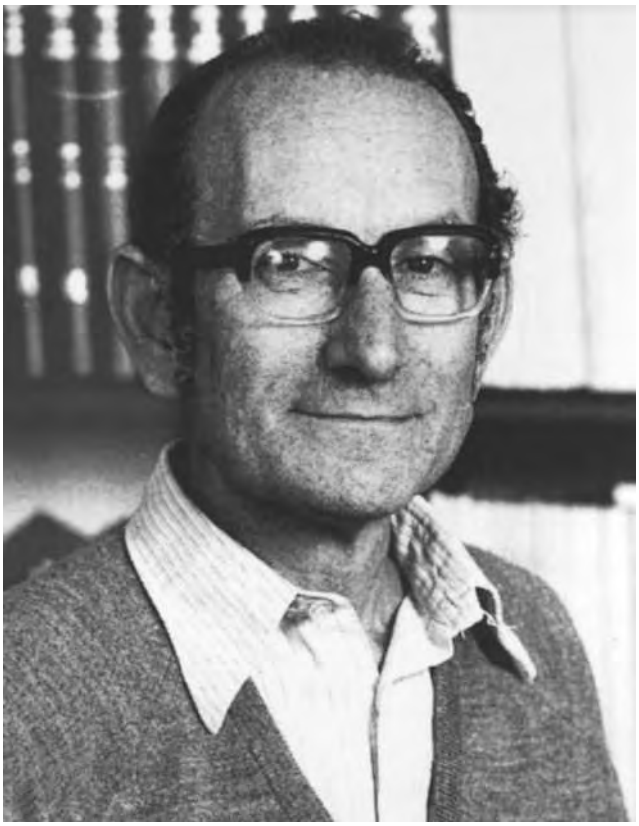
César Milstein conducted one of the most important late twentieth century studies on antibodies. In 1984, Milstein received the Nobel Prize for physiology or medicine, shared with **Niels K. Jerne** and **Georges Köhler**, for his outstanding contributions to **immunology** and immunogenetics. Milstein's research on the structure of antibodies and their genes, through the investigation of **DNA** (**deoxyribonucleic acid**) and **ribonucleic acid** (**RNA**), has been fundamental for a better understanding of how the human **immune system** works.

Milstein was born on October 8, 1927, in the eastern Argentine city of Bahía Blanca, one of three sons of Lázaro and Máxima Milstein. He studied **biochemistry** at the National University of Buenos Aires from 1945 to 1952, graduating with a degree in chemistry. Heavily involved in opposing the policies of President Juan Peron and working part-time as a chemical analyst for a laboratory, Milstein barely managed to pass with poor grades. Nonetheless, he pursued graduate studies at the Instituto de Biología Química of the University of Buenos Aires and completed his doctoral dissertation on the chemistry of aldehyde dehydrogenase, an alcohol enzyme used as a catalyst, in 1957.

With a British Council scholarship, he continued his studies at Cambridge University from 1958 to 1961 under the guidance of Frederick Sanger, a distinguished researcher in the field of **enzymes**. Sanger had determined that an enzyme's functions depend on the arrangement of amino acids inside it. In 1960, Milstein obtained a Ph.D. and joined the Department of Biochemistry at Cambridge, but in 1961, he decided to return to his native country to continue his investigations as head of a newly created Department of **Molecular Biology** at the National Institute of Microbiology in Buenos Aires.

A military coup in 1962 had a profound impact on the state of research and on academic life in Argentina. Milstein resigned his position in protest of the government's dismissal of the Institute's director, Ignacio Pirosky. In 1963, he returned to work with Sanger in Great Britain. During the 1960s and much of the 1970s, Milstein concentrated on the study of antibodies, the protein organisms generated by the immune system to combat and deactivate antigens. Milstein's efforts were aimed at analyzing myeloma proteins, and then DNA and RNA. Myeloma, which are tumors in cells that produce antibodies, had been the subject of previous studies by Rodney R. Porter, **MacFarlane Burnet**, and **Gerald M. Edelman**, among others.

Milstein's investigations in this field were fundamental for understanding how antibodies work. He searched for **mutations** in laboratory cells of myeloma but faced innumerable difficulties trying to find antigens to combine with their anti-



César Milstein

bodies. He and Köhler produced a hybrid myeloma called hybridoma in 1974. This cell had the capacity to produce antibodies but kept growing like the cancerous cell from which it had originated. The production of monoclonal antibodies from these cells was one of the most relevant conclusions from Milstein and his colleague's research. The Milstein-Köhler paper was first published in 1975 and indicated the possibility of using monoclonal antibodies for testing antigens. The two scientists predicted that since it was possible to hybridize antibody-producing cells from different origins, such cells could be produced in massive cultures. They were, and the technique consisted of a fusion of antibodies with cells of the myeloma to produce cells that could perpetuate themselves, generating uniform and pure antibodies.

In 1983, Milstein assumed leadership of the Protein and Nucleic Acid Chemistry Division at the Medical Research Council's laboratory. In 1984, he shared the Nobel Prize with Köhler and Jerne for developing the technique that had revolutionized many diagnostic procedures by producing exceptionally pure antibodies. Upon receiving the prize, Milstein heralded the beginning of what he called "a new era of immunobiochemistry," which included production of molecules based on antibodies. He stated that his method was a by-product of basic research and a clear example of how an investment in research that was not initially considered commercially viable had "an enormous practical impact." By 1984, a thriving business was being done with monoclonal antibodies for diagnosis, and works on vaccines and cancer based on Milstein's breakthrough research were being rapidly developed.

In the early 1980s, Milstein received a number of other scientific awards, including the Wolf Prize in Medicine from the Karl Wolf Foundation of Israel in 1980, the Royal Medal from the Royal Society of London in 1982, and the Dale Medal from the Society for Endocrinology in London in 1984. He was a member of numerous international scientific organizations, among them the U.S. National Academy of Sciences and the Royal College of Physicians in London.

See also Antibody and antigen; Antibody formation and kinetics; Antibody, monoclonal; Antibody-antigen, biochemical and molecular reactions

Minimum Inhibitory Concentration (MIC) • *see* Antibiotics

Mitochondria and cellular energy

Mitochondria are cellular organelles found in the **cytoplasm** in round and elongated shapes, that produce adenosine tri-phosphate (ATP) near intra-cellular sites where energy is needed. Shape, amount, and intra-cellular position of mitochondria are not fixed, and their movements inside cells are influenced by the cytoskeleton, usually in close relationship with the energetic demands of each cell type. For instance, cells that have a high consumption of energy, such as muscular, neural, retinal, and gonadic cells present much greater amounts of mitochondria than those with a lower energetic demand, such as fibroblasts and lymphocytes. Their position in cells also varies, with larger concentrations of mitochondria near the intra-cellular areas of higher energy consumption. In cells of the ciliated epithelium for instance, a greater number of mitochondria is found next to the cilia, whereas in spermatozoids they are found in greater amounts next to the initial portion of the flagellum, where the flagellar movement starts.

Mitochondria have their own **DNA**, **RNA** (rRNA, mRNA and tRNA) and **ribosomes**, and are able to synthesize proteins independently from the cell **nucleus** and the cytoplasm. The internal mitochondrial membrane contains more than 60 proteins. Some of these are **enzymes** and other proteins that constitute the electron-transporting chain; others constitute the elementary corpuscle rich in ATP-synthetase, the enzyme that promotes the coupling of electron transport to the synthesis of ATP; and finally, the enzymes involved in the active transport of substances through the internal membrane.

The main ultimate result of **respiration** is the generation of cellular energy through oxidative phosphorilation, i.e., ATP formation through the transfer of electrons from nutrient molecules to molecular oxygen. Prokaryotes, such as **bacteria**, do not contain mitochondria, and the flow of electrons and the oxidative phosphorilation process are associated to the internal membrane of these unicellular organisms. In eukaryotic

cells, the oxidative phosphorilation occurs in the mitochondria, through the chemiosmotic coupling, the process of transferring hydrogen protons (H^+) from the space between the external and the internal membrane of mitochondria to the elementary corpuscles. H^+ are produced in the mitochondrial matrix by the citric acid cycle and actively transported through the internal membrane to be stored in the inter-membrane space, thanks to the energy released by the electrons passing through the electron-transporting chain. The transport of H^+ to the elementary corpuscles is mediated by enzymes of the ATPase family and causes two different effects. First, 50% of the transported H^+ is dissipated as heat. Second, the remaining hydrogen cations are used to synthesize ATP from ADP (adenosine di-phosphate) and inorganic phosphate, which is the final step of the oxidative phosphorilation. ATP constitutes the main source of chemical energy used by the **metabolism** of eukaryotic cells in the activation of several multiple signal **transduction** pathways to the nucleus, intracellular enzymatic system activation, active transport of nutrients through the cell membrane, and nutrient metabolization.

See also Cell membrane transport; Krebs cycle; Mitochondrial DNA; Mitochondrial inheritance

MITOCHONDRIAL DNA

Mitochondria are cellular organelles that generate energy in the form of ATP through oxidative phosphorylation. Each cell contains hundreds of these important organelles. Mitochondria are inherited at conception from the mother through the **cytoplasm** of the egg. The mitochondria, present in all of the cells of the body, are copies of the ones present in at conception in the egg. When cells divide, the mitochondria that are present are randomly distributed to the daughter cells, and the mitochondria themselves then replicate as the cells grow.

Although many of the mitochondrial genes necessary for ATP production and other genes needed by the mitochondria are encoded in the **DNA** of the **chromosomes** in the **nucleus** of the cell, some of the genes expressed in mitochondria are encoded in a small circular chromosome which is contained within the mitochondrion itself. This includes 13 polypeptides, which are components of oxidative phosphorylation **enzymes**, 22 transfer **RNA** (t-RNA) genes, and two genes for ribosomal RNA (r-RNA). Several copies of the mitochondrial chromosome are found in each mitochondrion. These chromosomes are far smaller than the chromosomes found in the nucleus, contain far fewer genes than any of the autosomes, replicate without going through a mitotic cycle, and their morphological structure is more like a bacterial chromosome than it is like the chromosomes found in the nucleus of **eukaryotes**.

Genes which are transmitted through the mitochondrial DNA are inherited exclusively from the mother, since few if any mitochondria are passed along from the sperm. Genetic diseases involving these genes show a distinctive pattern of inheritance in which the trait is passed from an affected female to all of her children. Her daughters will likewise pass the trait on to all of her children, but her sons do not transmit the trait at all.

The types of disorders which are inherited through **mutations** of the mitochondrial DNA tend to involve disorders of nerve function, as neurons require large amounts of energy to function properly. The best known of the mitochondrial disorders is Leber hereditary optic neuropathy (LHON), which involves bilateral central vision loss, which quickly worsens as a result of the death of the optic nerves in early adulthood. Other mitochondrial diseases include Kearns-Sayre syndrome, myoclonus epilepsy with ragged red fibers (MERFF), and mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS).

See also Mitochondria and cellular energy; Mitochondrial inheritance; Ribonucleic acid (RNA)

MITOCHONDRIAL INHERITANCE

Mitochondrial inheritance is the study of how mitochondrial genes are inherited. Mitochondria are cellular organelles that contain their own **DNA** and **RNA**, allowing them to grow and replicate independent of the cell. Each cell has 10,000 mitochondria each containing two to ten copies of its genome. Because mitochondria are organelles that contain their own genome, they follow an inheritance pattern different from simple Mendelian inheritance, known as extranuclear or cytoplasmic inheritance. Although they posses their own genetic material, mitochondria are semi-autonomous organelles because the nuclear genome of cells still codes for some components of mitochondria.

Mitochondria are double membrane-bound organelles that function as the energy source of eukaryotic cells. Within the inner membrane of mitochondria are folds called cristae that enclose the matrix of the organelle. The DNA of mitochondria, located within the matrix, is organized into circular duplex **chromosomes** that lack histones and code for proteins, rRNA, and tRNA. A nucleoid, rather than a nuclear envelope, surrounds the genetic material of the organelle. Unlike the DNA of nuclear genes, the genetic material of mitochondria does not contain introns or repetitive sequences resulting in a relatively simple structure. Because the chromosomes of mitochondria are similar to those of prokaryotic cells, scientists hold that mitochondria evolved from free-living, aerobic **bacteria** more than a billion years ago. It is hypothesized that mitochondria were engulfed by eukaryotic cells to establish a symbiotic relationship providing metabolic advantages to each.

Mitochondria are able to divide independently without the aid of the cell. The chromosomes of mitochondria are replicated continuously by the enzyme DNA polymerase, with each strand of DNA having different points of origin. Initially, one of the parental strands of DNA is displaced while the other parental strand is being replicated. When the copying of the first strand of DNA is complete, the second strand is replicated in the opposite direction. Mutation rates of mitochondria are much greater than that of nuclear DNA allowing mitochondria to evolve more rapidly than nuclear genes. The resulting **phe-**

notype (cell death, inability to generate energy, or a silent mutation that has no phenotypic effect) is dependent on the number and severity of **mutations** within tissues.

During fertilization, mitochondria within the sperm are excluded from the zygote, resulting in mitochondria that come only from the egg. Thus, mitochondrial DNA is inherited through the maternal lineage exclusively without any **recombination** of genetic material. Therefore, any trait coded for by mitochondrial genes will be inherited from mother to all of her offspring. From an evolutionary standpoint, Mitochondrial Eve represents a single female ancestor from who our mitochondrial genes, not our nuclear genes, were inherited 200,000 years ago. Other women living at that time did not succeed in passing on their mitochondria because their offspring were only male. Although the living descendants of those other females were able to pass on their nuclear genes, only Mitochondrial Eve succeeded in passing on her mitochondrial genes to humans alive today.

See also Mitochondria and cellular energy; Mitochondrial DNA; Molecular biology and molecular genetics; Molecular biology, central dogma of

MOLD

Mold is the general term given to a coating or discoloration found on the surface of certain materials; it is produced by the growth of a fungus. Mold also refers to the causative organism itself.

A mold is a microfungus (as opposed to the macrofungi, such as mushrooms and toadstools) that feeds on dead organic materials. Taxonomically, the molds belong to a group of true **fungi** known as the Ascomycotina. The characteristics of the Ascomycotina are that their spores, that is their reproductive propagules (the fungal equivalent of seeds), are produced inside a structure called an ascus (plural asci). The spores are usually developed eight per ascus, but there are many asci per fruiting body (structures used by the fungus to produce and disperse the spores). A fruiting body of the Ascomycotina is properly referred to as an ascomata. Another characteristic of molds is their rapid growth once suitable conditions are encountered. They can easily produce a patch visible to the naked eye within one day.

The visible appearance of the mold can be of a soft, velvety pad or cottony mass of fungal tissue. If closely observed, the mass can be seen to be made up of a dense aggregation of thread-like mycelia (singular, **mycelium**) of the fungus. Molds can be commonly found on dead and decaying organic material, including improperly stored food stuffs.

The type of mold can be identified by its color and the nature of the substrate on which it is growing. One common example is white bread mold, caused by various species of the genera *Mucor* and *Rhizobium.* Citrus fruits often have quite distinctive blue and green molds of *Penicillium.* Because of the damages this group can cause, they are an economically important group.

In common with the other fungi, the molds reproduce by means of microscopic spores. These tiny spores are easily spread by even weak air currents, and consequently very few places are free of spores due to the astronomical number of spores a single ascomata can produce. Once a spore has landed on a suitable food supply, it requires the correct atmospheric conditions, i.e., a damp atmosphere, to germinate and grow.

Some molds such as *Mucor* and its close relatives have a particularly effective method of a sexual reproduction. A stalked structure is produced, which is topped by a clear, spherical ball with a black disc, within which the spores are developed and held. The whole structure is known as a sporangium (plural, sporangia). Upon maturity, the disc cracks open and releases the spores, which are spread far and wide by the wind. Some other molds, such as *Pilobolus,* fire their spores off like a gun and they land as a sticky mass up to 3 ft (1 m) away. Most of these never grow at all, but due to the vast number produced, up to 100,000 in some cases, this is not a problem for the fungus. As has already been mentioned, these fungi will grow on organic materials, including organic matter found within soil, so many types of molds are present in most places.

When sexual reproduction is carried out, each of the molds require a partner, as they are not capable of self-fertilization. This sexual process is carried out when two different breeding types grow together, and then swap haploid nuclei (containing only half the normal number of **chromosomes**), which then fuse to produce diploid zygospores (a thick-walled cell with a full number of chromosomes). These then germinate and grow into new colonies.

The *Mucor* mold, when grown within a closed environment, has mycelia that are thickly covered with small droplets of water. These are, in fact, diluted solutions of secondary metabolites. Some of the products of mold **metabolism** have great importance.

Rhizopus produces fumaric acid, which can be used in the production of the drug cortisone. Other molds can produce alcohol, citric acid, oxalic acid, or a wide range of other chemicals. Some molds can cause fatal neural diseases in humans and other animals.

Moldy bread is nonpoisonous. Nevertheless, approximately one hundred million loaves of moldy bread are discarded annually in the United States. The molds typically cause spoilage rather than rendering the bread poisonous. Some molds growing on food are believed to cause cancer, particularly of the liver. Another curious effect of mold is related to old, green wallpaper. In the nineteenth century, wallpaper of this color was prepared using compounds of arsenic, and when molds grow on this substrate, they have been known to release arsenic gas.

The first poison to be isolated from a mold is aflatoxin. This and other poisonous substances produced by molds and other fungi are referred to as mycotoxins. Some mycotoxins are deadly to humans in tiny doses, others will only affect certain animals. Aflatoxin was first isolated in 1960 in Great Britain. It was produced by *Aspergillus flavus* that had been growing on peanuts. In that year, aflatoxin had been responsible for the death of 100,000 turkeys—a massive financial loss that led to the research that discovered aflatoxin. From the

beginning of the twentieth century, scientists had tentatively linked a number of diseases with molds, but had not been able to isolate the compounds responsible. With the discovery of aflatoxin, scientists were able to provide proof of the undesirable effects of a mold.

Just because a particular mold can produce a mycotoxin does not mean it always will. For example, *Aspergillus flavus* has been safely used for many centuries in China in the production of various cheeses and soy sauce. *Aspergillus flavus* and related species are relatively common, and will grow on a wide variety of substrates, including various food-stuffs and animal feeds. However, the optimum conditions for vegetative growth are different from those required for the production of aflatoxin. The mycotoxin in this species is produced in largest quantities at high moisture levels and moderate temperatures on certain substrates. For a damaging amount of the toxin to accumulate, about ten days at these conditions may be required. Aflatoxin can be produced by *A. flavus* growing on peanuts. However, *A. flavus* will grow on cereal grains (such as wheat, corn, barley, etc.), but the mycotoxin is not produced on these growth media. Aflatoxin production is best prevented by using appropriate storage techniques.

Other molds can produce other mycotoxins, which can be just as problematical as aflatoxin. The term mycotoxin can also include substances responsible for the death of **bacteria**, although these compounds are normally referred to as **antibiotics**.

The molds do not only present humans with problems. Certain types of cheeses are ripened by mold fungi. Indeed, the molds responsible for this action have taken their names from the cheeses they affect. Camembert is ripened by *Penicillium camemberti,* and Roquefort is by *P. roqueforti.*

The *Pencillium* mold have another important use—the production of antibiotics. Two species have been used for the production of **penicillin**, the first antibiotic to be discovered: *Penicillium notatum* and *P. chrysogenum.* The *Penicillium* species can grow on different substrates, such as plants, cloth, leather, paper, wood, tree bark, cork, animal dung, carcasses, ink, syrup, seeds, and virtually any other item that is organic.

A characteristic that this mold does not share with many other species is its capacity to survive at low temperatures. Its growth rate is greatly reduced, but not to the extent of its competition, so as the temperature rises the *Penicillium* is able to rapidly grow over new areas. However, this period of initial growth can be slowed by the presence of other, competing **microorganisms**. Most molds will have been killed by the cold, but various bacteria may still be present. By releasing a chemical into the environment capable of destroying these bacteria, the competition is removed and growth of the *Penicillium* can carry on. This bacteria killing chemical is now recognized as penicillin.

The anti-bacterial qualities of penicillin were originally discovered by Sanford Fleming in 1929. By careful **selection** of the *Penicillium* cultures used, the yield of antibiotic has been increased many hundred fold since the first attempts of commercial scale production during the 1930s.

Other molds are used in various industrial processes. *Aspergillus* terreus is used to manufacture icatonic acid, which is used in plastics production. Other molds are used in the production of alcohol, a process that utilizes *Rhizopus,* which can metabolize starch into glucose. The *Rhizopus* species can then directly ferment the glucose to give alcohol, but they are not efficient in this process, and at this point brewers **yeast** *(Saccharomyces cerevisiae)* is usually added to ferment the glucose much quicker. Other molds are used in the manufacture of flavorings and chemical additives for food stuffs.

Cheese production has already been mentioned. It is interesting to note that in previous times cheese was merely left in a place where mold production was likely to occur. However, in modern production cheeses are inoculated with a pure **culture** of the mold (some past techniques involved adding a previously infected bit of cheese). Some of the mold varieties used in cheese production are domesticated, and are not found in the wild. In cheese production, the cultures are frequently checked to ensure that no **mutants** have arisen, which could produce unpalatable flavors.

Some molds are important crop **parasites** of species such as corn and millet. A number of toxic molds grow on straw and are responsible for diseases of livestock, including facial eczema in sheep, and slobber syndrome in various grazing animals. Some of the highly toxic chemicals are easy to identify and detect; others are not. Appropriate and sensible storage conditions, i.e., those not favoring the growth of fungi, are an adequate control measure in most cases. If mold is suspected then the use of anti fungal agents (**fungicides**) or destruction of the infected straw are the best options.

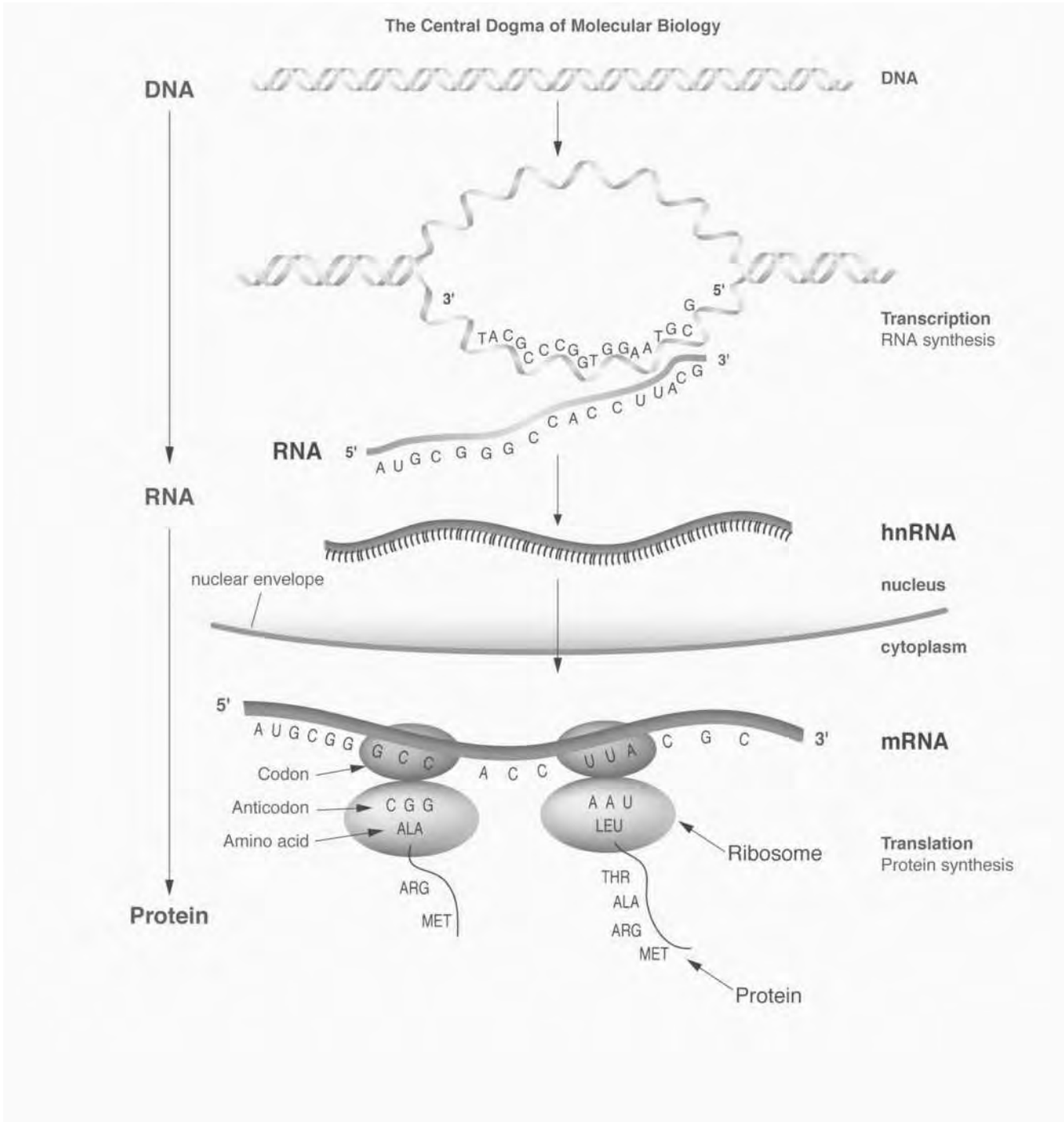
See also Fermentation; Food preservation; Food safety; Mycology; Yeast genetics; Yeast, infectious

MOLECULAR BIOLOGY AND MOLECULAR GENETICS

At its most fundamental level, molecular biology is the study of biological molecules and the molecular basis of structure and function in living organisms.

Molecular biology is an interdisciplinary approach to understanding biological functions and regulation at the level of molecules such as nucleic acids, proteins, and carbohydrates. Following the rapid advances in biological science brought about by the development and advancement of the Watson-Crick model of **DNA** (**deoxyribonucleic acid**) during the 1950s and 1960s, molecular biologists studied **gene** structure and function in increasing detail. In addition to advances in understanding genetic machinery and its regulation, molecular biologists continue to make fundamental and powerful discoveries regarding the structure and function of cells and of the mechanisms of genetic transmission. The continued study of these processes by molecular biologists and the advancement of molecular biological techniques requires integration of knowledge derived from physics, microbiology, mathematics, genetics, **biochemistry**, cell biology and other scientific fields.

Molecular biology also involves organic chemistry, physics, and biophysical chemistry as it deals with the physic-



The central dogma of molecular biology, DNA to RNA to protein.

ochemical structure of macromolecules (nucleic acids, proteins, lipids, and carbohydrates) and their interactions. Genetic materials including DNA in most of the living forms or **RNA** (**ribonucleic acid**) in all **plant viruses** and in some animal **viruses** remain the subjects of intense study.

The complete set of genes containing the genetic instructions for making an organism is called its genome. It contains the master blueprint for all cellular structures and activities for the lifetime of the cell or organism. The human genome consists of tightly coiled threads of deoxyribonucleic acid (DNA) and associated protein molecules organized into structures called **chromosomes**. In humans, as in other higher organisms, a DNA molecule consists of two strands that wrap around each other to resemble a twisted ladder whose sides,

made of sugar and phosphate molecules are connected by rungs of nitrogen-containing chemicals called bases (nitrogenous bases). Each strand is a linear arrangement of repeating similar units called nucleotides, which are each composed of one sugar, one phosphate, and a nitrogenous base. Four different bases are present in DNA adenine (A), thymine (T), cytosine (C), and guanine (G). The particular order of the bases arranged along the sugar-phosphate backbone is called the DNA sequence; the sequence specifies the exact genetic instructions required to create a particular organism with its own unique traits.

Each time a cell divides into two daughter cells, its full genome is duplicated; for humans and other complex organisms, this duplication occurs in the **nucleus**. During cell division the DNA molecule unwinds and the weak bonds between the base pairs break, allowing the strands to separate. Each strand directs the synthesis of a complementary new strand, with free nucleotides matching up with their complementary bases on each of the separated strands. Nucleotides match up according to strict base-pairing rules. Adenine will pair only with thymine (an A-T pair) and cytosine with guanine (a C-G pair). Each daughter cell receives one old and one new DNA strand. The cell's adherence to these base-pairing rules ensures that the new strand is an exact copy of the old one. This minimizes the incidence of errors (**mutations**) that may greatly affect the resulting organism or its offspring.

Each DNA molecule contains many genes, the basic physical and functional units of heredity. A gene is a specific sequence of nucleotide bases, whose sequences carry the information required for constructing proteins, which provide the structural components of cells and as well as **enzymes** for essential biochemical reactions.

The chromosomes of prokaryotic **microorganisms** differ from eukaryotic microorganisms, in terms of shape and organization of genes. Prokaryotic genes are more closely packed and are usually is arranged along one circular chromosome.

The central dogma of molecular biology states that DNA is copied to make mRNA (messenger RNA), and mRNA is used as the template to make proteins. Formation of RNA is called **transcription** and formation of protein is called **translation**. Transcription and translation processes are regulated at various stages and the regulation steps are unique to prokaryotes and **eukaryotes**. DNA regulation determines what type and amount of mRNA should be transcribed, and this subsequently determines the type and amount of protein. This process is the fundamental control mechanism for growth and development (morphogenesis).

All living organisms are composed largely of proteins, the end product of genes. Proteins are large, complex molecules made up of long chains of subunits called amino acids. The protein-coding instructions from the genes are transmitted indirectly through messenger ribonucleic acid (mRNA), a transient intermediary molecule similar to a single strand of DNA. For the information within a gene to be expressed, a complementary RNA strand is produced (a process called transcription) from the DNA template. In eukaryotes, messenger RNA (mRNA) moves from the nucleus to the cellular **cytoplasm**, but in both eukaryotes and prokaryotes mRNA serves as the template for **protein synthesis**.

Twenty different kinds of amino acids are usually found in proteins. Within the gene, sequences of three DNA bases serve as the template for the construction of mRNA with sequence complimentary codons that serve as the language to direct the cell's protein-synthesizing machinery. Cordons specify the insertion of specific amino acids during the synthesis of protein. For example, the base sequence ATG codes for the amino acid methionine. Because more than one codon sequence can specify the same amino acid, the **genetic code** is termed a degenerate code (i.e., there is not a unique codon sequence for every amino acid).

Areas of intense study by molecular biology include the processes of DNA replication, repair, and mutation (alterations in base sequence of DNA). Other areas of study include the identification of agents that cause mutations (e.g., ultra-violet rays, chemicals) and the mechanisms of rearrangement and exchange of genetic materials (e.g. the function and control of small segments of DNA such as **plasmids**, transposable elements, **insertion sequences**, and **transposons** to obtain recombinant DNA).

Recombinant DNA technologies and genetic engineering are an increasingly important part of molecular biology. Advances in **biotechnology** and molecular medicine also carry profound clinical and social significance. Advances in molecular biology have led to significant discoveries concerning the mechanisms of the embryonic development, disease, immunologic response, and **evolution**.

See also Immunogenetics; Microbial genetics

MONOCLONAL ANTIBODY • *see* ANTIBODY, MONOCLONAL

MONOD, JACQUES LUCIEN (1910-1976)

French biologist

French biologist Jacques Lucien Monod and his colleagues demonstrated the process by which messenger **ribonucleic acid** (mRNA) carries instructions for **protein synthesis** from **deoxyribonucleic acid** (**DNA**) in the cell **nucleus** out to the **ribosomes** in the **cytoplasm**, where the instructions are carried out.

Jacques Monod was born in Paris. In 1928, Monod began his study of the natural sciences at the University of Paris, Sorbonne where he went on to receive a B.S. from the *Faculte des Sciences* in 1931. Although he stayed on at the university for further studies, Monod developed further scientific grounding during excursions to the nearby Roscoff marine biology station.

While working at the Roscoff station, Monod met André Lwoff, who introduced him to the potentials of microbiology and microbial nutrition that became the focus of Monod's early research. Two other scientists working at Roscoff station, Boris Ephrussi and Louis Rapkine, taught Monod the

importance of physiological and biochemical genetics and the relevance of learning the chemical and molecular aspects of living organisms, respectively.

During the autumn of 1931, Monod took up a fellowship at the University of Strasbourg in the laboratory of Edouard Chatton, France's leading protistologist. In October 1932, he won a Commercy Scholarship that called him back to Paris to work at the Sorbonne once again. This time he was an assistant in the Laboratory of the **Evolution** of Organic Life, which was directed by the French biologist Maurice Caullery. Moving to the zoology department in 1934, Monod became an assistant professor of zoology in less than a year. That summer, Monod also embarked on a natural history expedition to Greenland aboard the *Pourquoi pas?* In 1936, Monod left for the United States with Ephrussi, where he spent time at the California Institute of Technology on a Rockefeller grant. His research centered on studying the fruit fly (*Drosophila melanogaster*) under the direction of Thomas Hunt Morgan, an American geneticist. Here Monod not only encountered new opinions, but he also had his first look at a new way of studying science, a research style based on collective effort and a free passage of critical discussion. Returning to France, Monod completed his studies at the Institute of Physiochemical Biology. In this time he also worked with Georges Teissier, a scientist at the Roscoff station who influenced Monod's interest in the study of **bacterial growth**. This later became the subject of Monod's doctoral thesis at the Sorbonne where he obtained his Ph.D. in 1941.

Monod's work comprised four separate but interrelated phases beginning with his practical education at the Sorbonne. In the early years of his education, he concentrated on the kinetic aspects of biological systems, discovering that the growth rate of **bacteria** could be described in a simple, quantitative way. The size of the **colony** was solely dependent on the food supply; the more sugar Monod gave the bacteria to feed on, the more they grew. Although there was a direct correlation between the amounts of food Monod fed the bacteria and their rate of growth, he also observed that in some colonies of bacteria, growth spread over two phases, sometimes with a period of slow or no growth in between. Monod termed this phenomenon *diauxy* (double growth), and guessed that the bacteria had to employ different **enzymes** to metabolize different kinds of sugars.

When Monod brought the finding to Lwoff's attention in the winter of 1940, Lwoff suggested that Monod investigate the possibility that he had discovered a form of enzyme adaptation, in that the latency period represents a hiatus during which the colony is switching between enzymes. In the previous decade, the Finnish scientist, Henning Karstroem, while working with protein synthesis had recorded a similar phenomenon. Although the outbreak of war and a conflict with his director took Monod away from his lab at the Sorbonne, Lwoff offered him a position in his laboratory at the Pasteur Institute where Monod would remain until 1976. Here he began working with Alice Audureau to investigate the genetic consequences of his kinetic findings, thus beginning the second phase of his work.

To explain his findings with bacteria, Monod shifted his focus to the study of **enzyme induction**. He theorized that certain colonies of bacteria spent time adapting and producing enzymes capable of processing new kinds of sugars. Although this slowed down the growth of the colony, Monod realized that it was a necessary process because the bacteria needed to adapt to varying environments and foods to survive. Therefore, in devising a mechanism that could be used to sense a change in the environment, and thereby enable the colony to take advantage of the new food, a valuable evolutionary step was taking place. In Darwinian terms, this colony of bacteria would now have a very good chance of surviving, by passing these changes on to future generations. Monod summarized his research and views on relationship between the roles of random chance and adaptation in evolution in his 1970 book *Chance and Necessity.*

Between 1943 and 1945, working with Melvin Cohn, a specialist in **immunology**, Monod hit upon the theory that an inducer acted as an internal signal of the need to produce the required digestive enzyme. This hypothesis challenged the German biochemist Rudolf Schoenheimer's theory of the dynamic state of protein production that stated it was the mix of proteins that resulted in a large number of random combinations. Monod's theory, in contrast, projected a fairly stable and efficient process of protein production that seemed to be controlled by a master plan. In 1953, Monod and Cohn published their findings on the generalized theory of induction.

That year Monod also became the director of the department of cellular biology at the Pasteur Institute and began his collaboration with **François Jacob**. In 1955, working with Jacob, he began the third phase of his work by investigating the relationship between the roles of heredity and environment in enzyme synthesis, that is, how the organism creates these vital elements in its metabolic pathway and how it knows when to create them.

It was this research that led Monod and Jacob to formulate their model of protein synthesis. They identified a **gene** cluster they called the **operon**, at the beginning of a strand of bacterial DNA. These genes, they postulated, send out messages signaling the beginning and end of the production of a specific protein in the cell, depending on what proteins are needed by the cell in its current environment. Within the operons, Monod and Jacob discovered two key genes, which they named the operator and structural genes. The scientists discovered that during protein synthesis, the operator gene sends the signal to begin building the protein. A large molecule then attaches itself to the structural gene to form a strand of mRNA. In addition to the operon, the regulator gene codes for a repressor protein. The repressor protein either attaches to the operator gene and inactivates it, in turn, halting structural gene activity and protein synthesis; or the repressor protein binds to the regulator gene instead of the operator gene, thereby freeing the operator and permitting protein synthesis to occur. As a result of this process, the mRNA, when complete, acts as a template for the creation of a specific protein encoded by the DNA, carrying instructions for protein synthesis from the DNA in the cell's nucleus, to the ribosomes outside the nucleus, where proteins are manufactured. With such a system, a cell can adapt to changing environmental conditions, and produce the proteins it needs when it needs them.

Word of the importance of Monod's work began to spread, and in 1958 he was invited to become professor of **biochemistry** at the Sorbonne, a position he accepted conditional to his retaining his post at the Pasteur Institute. At the Sorbonne, Monod was the chair of chemistry of **metabolism**, but in April 1966, his position was renamed the chair of **molecular biology** in recognition of his research in creating the new science. Monod, Jacob, Lwoff won the 1965 Nobel Prize for physiology or medicine for their discovery of how genes regulate cell metabolism.

See also Bacterial growth and division; Microbial genetics; Molecular biology and molecular genetics

MONONUCLEOSIS, INFECTIOUS

Infectious mononucleosis is an illness caused by the **Epstein-Barr virus**. The symptoms of "mono," as the disease is colloquially called, include extreme fatigue, fever, sore throat, enlargement of the lymph nodes in the neck, armpit, and throat, sore muscles, loss of appetite, and an enlarged spleen. More infrequently, an individual will experience nausea, **hepatitis**, jaundice (which indicates malfunction of the liver), severe headache, chest pain, and difficulty breathing. Children may display only a few or none of these symptoms, while all can be present in adolescents.

The illness can be passed from person to person via the saliva. In adolescents, mononucleosis was once known as "the kissing disease" since kissing is a route of transmission of the Epstein-Barr virus. Given the relative ease of transmissions, epidemic outbreaks of mononucleosis can occur in environments such as schools, hospitals and the workplace.

Infectious mononucleosis is usually self-limiting. Recovery occurs with time and rest, and is usually complete with no after effects. Analgesics can help relieve the symptoms of pain and fever in adults. However, children should avoid taking aspirin, as use of the drug in viral illnesses is associated with the development of Reye syndrome, which can cause liver failure and even death.

Recovery from mononucleosis is not always complete. In some people there can be a decrease in the number of red and white blood cells, due either to damage to the bone marrow (where the blood cells are produced) or to enhanced destruction of the red blood cells (a condition known as hemolytic anemia). Another temporary complication of the illness is weakened or paralyzed facial muscles on one side of the face. The condition, which is called Bell's palsy, leaves the individual with a drooping look to one side of the face. Much more rarely, very severe medical complications can arise. These include rupture of the spleen, swelling of the heart (myocarditis), malfunction of the central nervous system, and Guillain-Barré syndrome. The latter condition is a paralysis resulting from disruption of nervous system function.

The illness is diagnosed in a number of ways. Clinically, the presence of fever, and **inflammation** of the pharynx and the lymph nodes are hallmarks of the illness. Secondly, the so-called "mono spot" test will demonstrate an elevated amount of **antibody** to the virus in the bloodstream. A third diagnostic feature of the illness is an increase in the number of white blood cells. These cells, which are also called lymphocytes, help fight viral infections.

Antibodies to the Epstein-Barr virus persist for a long time. Therefore, one bout of the illness usually bestows long-lasting **immunity** in an individual. Testing has demonstrated that most people have antibodies to the Epstein-Barr virus. Thus, most people have been infected with the virus at some point in their lives, but have displayed only a few minor symptoms or no symptoms at all. Many children are infected with the virus and either display no symptoms or become transiently ill with one of the retinue of infections acquired during the first few years of life. When the initial infection occurs during adolescence, the development of mononucleosis results 35–50% of the time. Understanding of the reasons for this failure to infect could lead to a **vaccine** to prevent infectious mononucleosis. As of 2002, there is no vaccine available.

The Epstein-Barr virus that is responsible for the illness is a member of the herpesvirus family. The virus is found all over the world and is one of the most common human **viruses**. In infectious mononucleosis, the virus infects and makes new copies of itself in the epithelial cells of the oropharynx. Also, the virus invades the **B cells** of the **immune system**.

For most patients, the infection abates after two to four weeks. Several more weeks may pass before the spleen resumes its normal size. A period of low activity is usually prescribed after a bout of mononucleosis, to protect the spleen and to help energy levels return to normal.

Epstein-Barr virus is usually still present after an infection has ended. The virus becomes dormant in some cells of the throat, in the blood, and in some cells of the immune system. Very rarely in some individuals, the latent virus may be linked to the appearance years later of two types of cancers; Burkitt's lymphoma and nasopharyngeal carcinoma.

See also Viruses and responses to viral infection

MONTAGNIER, LUC (1932-)

French virologist

Luc Montagnier, Distinguished Professor at Queens College in New York and the Institut Pasteur in Paris, has devoted his career to the study of **viruses**. He is perhaps best known for his 1983 discovery of the **human immunodeficiency virus** (**HIV**), which has been identified as the cause of acquired **immunodeficiency** syndrome (**AIDS**). However, in the twenty years before the onset of the AIDS epidemic, Montagnier made many significant discoveries concerning the nature of viruses. He made major contributions to the understanding of how viruses can alter the genetic information of host organisms, and significantly advanced cancer research. His investigation of interferon, one of the body's defenses against viruses, also opened avenues for medical cures for viral diseases. Montagnier's ongoing research focuses on the search for an AIDS **vaccine** or cure.

Luc Montagnier

Montagnier was born in Chabris (near Tours), France, the only child of Antoine Montagnier and Marianne Rousselet. He became interested in science in his early childhood through his father, an accountant by profession, who carried out experiments on Sundays in a makeshift laboratory in the basement of the family home. At age fourteen, Montagnier himself conducted nitroglycerine experiments in the basement laboratory. His desire to contribute to medical knowledge was also kindled by his grandfather's long illness and death from colon cancer.

Montagnier attended the Collège de Châtellerault, and then the University of Poitiers, where he received the equivalent of a bachelor's degree in the natural sciences in 1953. Continuing his studies at Poitiers and then at the University of Paris, he received his licence ès sciences in 1955. As an assistant to the science faculty at Paris, he taught physiology at the Sorbonne and in 1960, qualified there for his doctorate in medicine. He was appointed a researcher at the Centre National de la Recherche Scientifique (C.N.R.S.) in 1960, but then went to London for three and a half years to do research at the Medical Research Council at Carshalton.

Viruses are agents that consist of genetic material surrounded by a protective protein shell. They are completely dependent on the cells of a host animal or plant to multiply, a process that begins with the shedding of their own protein shell. The virus research group at Carshalton was investigating **ribonucleic acid** (**RNA**), a form of nucleic acid that normally is involved in taking genetic information from **deoxyribonucleic acid** (**DNA**) (the main carrier of genetic information) and translating it into proteins. Montagnier and F. K. Sanders, investigating viral RNA (a virus that carries its genetic material in RNA rather than DNA), discovered a double-stranded RNA virus that had been made by the replication of a single-stranded RNA. The double-stranded RNA could transfer its genetic information to DNA, allowing the virus to encode itself in the genetic make-up of the host organism. This discovery represented a significant advance in knowledge concerning viruses.

From 1963 to 1965, Montagnier did research at the Institute of **Virology** in Glasgow, Scotland. Working with Ian MacPherson, he discovered in 1964 that **agar**, a gelatinous extractive of a red alga, was an excellent substance for culturing cancer cells. Their technique became standard in laboratories investigating oncogenes (genes that have the potential to make normal cells turn cancerous) and cell transformations. Montagnier himself used the new technique to look for cancer-causing viruses in humans after his return to France in 1965.

From 1965 to 1972, Montagnier worked as laboratory director of the Institut de Radium (later called Institut Curie) at Orsay. In 1972, he founded and became director of the viral oncology unit of the Institut Pasteur. Motivated by his findings at Carshalton and the belief that some cancers are caused by viruses, Montagnier's basic research interest during those years was in **retroviruses** as a potential cause of cancer. Retroviruses possess an enzyme called reverse transcriptase. Montagnier established that reverse transcriptase translates the genetic instructions of the virus from the viral (RNA) form to DNA, allowing the genes of the virus to become permanently established in the cells of the host organism. Once established, the virus can begin to multiply, but it can do so only by multiplying cells of the host organism, forming malignant tumors. In addition, collaborating with Edward De Mayer and Jacqueline De Mayer, Montagnier isolated the messenger RNA of interferon, the cell's first defense against a virus. Ultimately, this research allowed the **cloning** of interferon genes in a quantity sufficient for research. However, despite widespread hopes for interferon as a broadly effective anticancer drug, it was initially found to be effective in only a few rare kinds of malignancies.

AIDS (acquired immunodeficiency syndrome), an epidemic that emerged in the early 1980s, was first adequately characterized around 1982. Its chief feature is that it disables the **immune system** by which the body defends itself against numerous diseases. It is eventually fatal. By 1993, more than three million people had developed AIDS. Montagnier considered that a retrovirus might be responsible for AIDS. Researchers had noted that one pre-AIDS condition involved a persistent enlargement of the lymph nodes, called lymphadenopathy. Obtaining some tissue **culture** from the lymph nodes of an infected patient in 1983, Montagnier and two colleagues, Françoise Barré-Sinoussi and Jean-Claude Chermann, searched for and found reverse transcriptase, which constitutes evidence of a retrovirus. They isolated a virus they called LAV (lymphadenopathy-associated virus). Later, by international agreement, it was renamed HIV, human immunodeficiency virus. After the virus had been isolated, it

was possible to develop a test for antibodies that had developed against it—the HIV test. Montagnier and his group also discovered that HIV attacks T4 cells, which are crucial in the immune system. A second similar but not identical HIV virus called HIV–2 was discovered by Montagnier and colleagues in April 1986.

A controversy developed over the patent on the HIV test in the mid–1980s. **Robert C. Gallo** of the National Cancer Institute in Bethesda, Maryland, announced his own discovery of the HIV virus in April 1984 and received the patent on the test. The Institut Pasteur claimed the patent (and the profits) based on Montagnier's earlier discovery of HIV. Despite the controversy, Montagnier continued research and attended numerous scientific meetings with Gallo to share information. Intense mediation efforts by **Jonas Salk** (the scientist who developed the first polio vaccine) led to an international agreement signed by the scientists and their respective countries in 1987. Montagnier and Gallo agreed to be recognized as co-discoverers of the virus, and the two governments agreed that the profits of the HIV test be shared most going to a foundation for AIDS research).

The scientific dispute continued to resurface, however. Most HIV viruses from different patients differ by six to twenty percent because of the remarkable ability of the virus to mutate. However, Gallo's virus was less than two percent different from Montagnier's, leading to the suspicion that both viruses were from the same source. The laboratories had exchanged samples in the early 1980s, which strengthened the suspicion. Charges of scientific misconduct on Gallo's part led to an investigation by the National Institutes of Health in 1991, which initially cleared Gallo. In 1992, the investigation was reviewed by the newly created Office of Research Integrity. The ORI report, issued in March of 1993, confirmed that Gallo had in fact "discovered" the virus sent to him by Montagnier. Whether Gallo had been aware of this fact in 1983 could not be established, but it was found that he had been guilty of misrepresentations in reporting his research and that his supervision of his research lab had been desultory. The Institut Pasteur immediately revived its claim to the exclusive right to the patent on the HIV test. Gallo objected to the decision by the ORI, however, and took his case before an appeals board at the Department of Health and Human Services. The board in December of 1993 cleared Gallo of all charges, and the ORI subsequently withdrew their charges for lack of proof.

More than a decade after setting the personal considerations aside, in May of 2002, the two scientists announced a partnership in the effort to speed the development of a vaccine against AIDS. Gallo will oversee research from the Institute of Human Virology, while Montagnier pursues concurrent research as head of the World Foundation for AIDS Research and Prevention in New York, Rome, and Paris.

Montagnier's continuing work includes investigation of the envelope proteins of the virus that link it to the T-cell. He is also extensively involved in research of possible drugs to combat AIDS. In 1990, Montagnier hypothesized that a second organism, called a mycoplasma, must be present with the HIV virus for the latter to become deadly. This suggestion, which has proved controversial among most AIDS researchers, is the subject of ongoing research.

Montagnier married Dorothea Ackerman in 1961. The couple has three children. He has described himself as an aggressive researcher who spends much time in either the laboratory or traveling to scientific meetings. Montagnier enjoys swimming and classical music, and loves to play the piano, especially Mozart sonatas.

See also AIDS, recent advances in research and treatment; Immunodeficiency diseases; Viruses and responses to viral infection

MONTAGUE, MARY WORTLEY (1689-1762)

English smallpox vaccination advocate

Lady Mary Wortley Montague contributed to microbiology and **immunology** by virtue of her powers of observation and her passion for letter writing. As the wife of the British Ambassador Extraordinary to the Turkish court, Montague and her family lived in Istanbul. While there she observed and was convinced of the protective power of inoculation against the disease **smallpox**. She wrote to friends in England describing inoculation and later, upon their return to England, she worked to popularize the practice of inoculation in that country.

Montague's interest in smallpox stemmed from her brush with the disease in 1715, which left her with a scarred face and lacking eyebrows, and also from the death of her brother from the disease. While posted in Istanbul, she was introduced to the practice of inoculation. Material picked from a smallpox scab on the surface of the skin was rubbed into an open cut of another person. The recipient would usually develop a mild case of smallpox but would never be ravaged by the full severity of the disease caused by more virulent strains of the smallpox virus. Lady Montague was so enthused by the protection offered against smallpox that she insisted on having her children inoculated. In 1718, her three-year-old son was inoculated. In 1721, having returned to England, she insisted that her English doctor inoculate her five-year-old daughter.

Upon her return to England following the expiration of her husband's posting, Montague used her standing in the high society of the day to promote the benefits of smallpox inoculation. Her passion convinced a number of English physicians and even the reigning Queen, who decreed that the royal children and future heirs to the crown would be inoculated against the disease. In a short time, it became fashionable to be one of those who had received an inoculation, partly perhaps because it was a benefit available only to the wealthy. Inoculation became a sign of status.

Smallpox outbreaks of the eighteenth century in England demonstrated the effectiveness of inoculation. The death rate among those who had been inoculated against smallpox was far less than among the uninoculated.

A few decades later, **Edward Jenner** refined the inoculation process by devising a **vaccine** for smallpox. History has

tended to credit Jenner with the discovery of a cure for smallpox. This is likely a reflection of the lack of credence given by the mostly male medical profession to the opinions of women. But there is no doubt that Jenner was aware of, and built upon, the inoculation strategy popularized by Lady Montague.

The receptiveness toward smallpox **vaccination** initially, and subsequently to a variety of vaccination strategies, stemmed from the efforts of Lady Montague. The acceptance of inoculation among the rich, powerful and influential of Europe led to the general acceptance of the practice among all sectors of society. With time, smallpox vaccination grew in worldwide popularity. So much so that in 1979, the United Nations **World Health Organization** declared that smallpox had been essentially eradicated. The pioneering efforts of Lady Montague have saved hundreds of millions of lives over the last 284 years.

See also Immunity, active, passive and delayed

Moore, Ruth Ella (1903-1994)

American bacteriologist

Ruth Ella Moore achieved distinction when she became the first African American woman to earn a Ph.D. in bacteriology from Ohio State in 1933. Her entire teaching career was spent at Howard University in Washington, D.C., where she remained an associate professor emeritus of microbiology until 1990.

Moore was born in Columbus, Ohio, on May 19, 1903. After receiving her B.S. from Ohio State in 1926, she continued at that university and received her M.A. the following year. In 1933 she earned her Ph.D. in bacteriology from Ohio State, becoming the first African American woman to do so. Her achievement was doubly significant considering that her minority status was combined with that era's social prejudices against women in professional fields. During her graduate school years (1927–1930), Moore was an instructor of both **hygiene** and English at Tennessee State College. Upon completing her dissertation at Ohio State—where she focused on the bacteriological aspects of **tuberculosis** (a major national health problem in the 1930s—she received her Ph.D.

Moore accepted a position at the Howard University College of Medicine as an instructor of bacteriology. In 1939 she became an assistant professor of bacteriology, and in 1948 she was named acting head of the university's department of bacteriology, preventive medicine, and **public health**. In 1955, she became head of the department of bacteriology and remained in that position until 1960 when she became an associate professor of microbiology at Howard. She remained in that department until her retirement in 1973, whereupon she became an associate professor emeritus of microbiology.

Throughout her career, Moore remained concerned with public health issues, and remained a member of the American Public Health Association and the American Society of Microbiologists.

See also History of microbiology; History of public health; Medical training and careers in microbiology

Most probable number (MPN) · *see*

Laboratory techniques in microbiology

Mumps

Mumps is a contagious viral disease that causes painful enlargement of the salivary glands, most commonly the parotids. Mumps is sometimes known as epidemic parotitis and occurs most often in children between the ages of 4 and 14.

Mumps was first described by Hippocrates (c.460–c.370 B.C.), who observed that the diseases occurred most commonly in young men, a fact that he attributed to their congregating at sports grounds. Women, who were inclined to be isolated in their own homes, were seldom taken ill with the disease. Over the centuries, medical writers paid little attention to mumps. Occasionally, mention was made of a local epidemic of the disease, as recorded in Paris, France, in the sixteenth century by Guillaume de Baillou (1538–1616). Most physicians believed that the disease was contagious, but no studies were made to confirm this suspicion. The first detailed scientific description of mumps was provided by the British physician Robert Hamilton (1721–1793) in 1790. Hamilton's paper in the Transactions of the Royal Society of Edinburgh finally made the disease well known among physicians. Efforts to prove the contagious nature of mumps date around 1913. In that year, two French physicians, Charles-Jean-Henri Nicolle (1866–1936) and Ernest Alfred Conseil, attempted to transmit mumps from humans to monkeys, but were unable to obtain conclusive results. Eight years later, Martha Wollstein injected **viruses** taken from the saliva of a mumps patient into cats, producing **inflammation** of the parotid, testes, and brain tissue in the cats. Conclusive proof that mumps is transmitted by a filterable virus was finally obtained by two American researchers, Claude D. Johnson and **Ernest William Goodpasture** (1886–1960), in 1934.

The mumps virus has an incubation period of 12-28 days with an average of 18 days. Pain and swelling in the region of one parotid gland, accompanied by some fever, is the characteristic initial presenting feature. About five days later, the other parotid gland may become affected while the swelling in the first gland has mainly subsided. In most children, the infection is mild and the swelling in the salivary glands usually disappears within two weeks. Occasionally, there is no obvious swelling of the glands during the infection. Children with mumps are infectious from days one to three before the parotid glands begin to swell, and remain so until about seven days after the swelling has disappeared. The disease can be transmitted through respiratory droplets. There are occasional complications in children with mumps. In the central nervous system (CNS), a rare complication is asceptic **meningitis** or encephalitis. This usually has an excellent prognosis. In about 20% of post-pubertal males, orchitis may arise as a complication and, rarely, can lead to sterility. A very rare additional complication is pancreatitis, which may require treatment and hospitalization.

The diagnosis of mumps in children is usually made on the basis of its very characteristic symptoms. The virus can be cultured, however, and can be isolated from a patient by taking a swab from the buccal (mouth) outlet of the parotid gland duct. The swab is then broken off into viral transport medium. **Culture** of the virus is rarely necessary in a straightforward case of mumps parotitis. Occasionally, it is necessary to isolate the virus from the cerebro-spinal fluid (CSF) of patients with CNS complications such as mumps meningitis. Also, serological investigations may be useful in aseptic meningitis and encephalitis.

A **vaccine** for mumps was developed by the American microbiologist, John Enders, in 1948. During World War II, Enders had developed a vaccine using a killed virus, but it was only moderately and temporarily successful. After the war, he began to investigate ways of growing mumps virus in a suspension of minced chick embryo and ox blood. The technique was successful and Enders' live virus vaccine is now routinely used to vaccinate children. In the U.S.A., the live attenuated mumps vaccine is sometimes given alone or together with **measles** and/or rubella vaccine. The MMR vaccine came under investigation with regard to a possible link to autism in children. The United States **Centers for Disease Control** concludes that current scientific evidence does not support any hypothesis that the MMR vaccine causes any form of autism. The hypothetical relationship, however, did discourage and continues to discourage some parents from allowing their children to receive the triple vaccine.

See also Antibody-antigen, biochemical and molecular reactions; History of immunology; History of public health; Immunity, active, passive and delayed; Immunology; Varicella; Viruses and responses to viral infection

MURCHISON METEORITE

The Murchison meteorite was a meteorite that entered Earth's atmosphere in September, 1969. The meteor fragmented before impact and remnants were recovered near Murchison, Australia (located about 60 miles north of Melbourne). The fragments recovered dated to nearly five billion years ago—to the time greater than the estimated age of Earth. In addition to interest generated by the age of the meteorite, analysis of fragments revealed evidence of carbon based compounds. The finds have fueled research into whether the organic compounds were formed from inorganic processes or are proof of extraterrestrial life dating to the time of Earth's creation.

In particular, it was the discovery of amino acids—and the percentages of the differing types of amino acids found (e.g., the number of left handed amino acids vs. right handed amino acids—that made plausible the apparent evidence of extraterrestrial organic processes, as opposed to biological **contamination** by terrestrial sources.

If the compounds prove to be from extraterrestrial life, this would constitute a profound discovery that would have far reaching global scientific and social impact concerning prevailing hypotheses concerning the **origin of life**. For example, some scientists, notably one of the discoverers of the structure of **DNA**, Sir **Francis Crick**, assert that in the period from the formation of Earth to the time of the deposition of the earliest discovered fossilized remains, there was insufficient time for evolutionary process to bring forth life in the abundance and variety demonstrated in the fossil record. Crick and others propose that a form of organic molecular "seeding" by meteorites exemplified by the Murchison meteorite (meteorites rich in complex carbon compounds) greatly reduced the time needed to develop life on Earth.

In fact, the proportions of the amino acids found in the Murchison meteorite approximated the proportions proposed to exist in the primitive atmosphere modeled in the **Miller-Urey experiment**. First conducted in 1953, University of Chicago researchers **Stanley L. Miller** and Harold C. Urey developed an experiment to test possible mechanisms in Earth's primitive atmosphere that could have produced organic molecules from inorganic processes. Methane (CH_4), hydrogen (H_2), and ammonia (NH_3) gases were introduced into a moist environment above a water-containing flask. To simulate primitive lightning discharges, Miller supplied the system with electrical current. Within days, organic compounds formed—including some amino acids. A classic experiment in **molecular biology**, the Miller-Urey experiment established that the conditions that existed in Earth's primitive atmosphere were sufficient to produce amino acids, the subunits of proteins comprising and required by living organisms. It is possible, however, that extraterrestrial organic molecules could have accelerated the formation of terrestrial organic molecules by serving as molecular templates.

In 1997, NASA scientists announced evidence that the Murchison meteorite contained microfossils that resemble **microorganisms**. The microfossils were discovered in fresh breaks of meteorite material. The potential finding remains the subject of intense scientific study and debate.

University of Texas scientists Robert Folk and F. Leo Lynch also announced the observation of fossils of terrestrial nanobacteria in another carbonaceous chondrite meteorite named the Allende meteorite. Other research has demonstrated that the Murchison and Murray meteorites (a carbonaceous chondrite meteorite found in Kentucky) contain sugars critical for the development of life.

See also Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Life, origin of

MUREIN • *see* PEPTIDOGLYCAN

MURRAY, ROBERT (1919-)

British bacteriologist

Robert George Everitt Murray is professor emeritus and former department chair of the Department of Microbiology and **Immunology** at the University of Western Ontario in London. His numerous accomplishments in bacterial taxonomy, ultra-

structure, and education have been recognized by his investiture as an officer of the Order of Canada in 1998.

Murray received his early education in Britain, but moved to Montreal in 1930 where his father was Professor of Bacteriology and Immunology at McGill University. He attended McGill from 1936 to 1938,then returned to England to study at Cambridge University (B.A. in Pathology and Bacteriology in 1941 and with a M.A. in the same discipline in 1945). In 1943 he also received a M.D. degree from McGill.

In 1945, Murray joined the faculty of the Department of Bacteriology and Immunology at the University of Western Ontario in London as a Lecturer. He remained at Western for the remainder of his career. He was appointed Professor and Head of the department in 1949 and served as head until 1974. Since his retirement in 1984 he has been Professor Emeritus.

Murray has served as President of the American Society for Microbiology in 1972–1973 and was one of the founders of the Canadian Society for Microbiologists in 1951. In 1954, he became the founding editor of the Canadian Journal of Microbiology, which continues to publish to this day.

His interest in taxonomy continued a family tradition begun by his father, E.G.D. Murray, who was a trustee of the Bergey's Manual of determinative Bacteriology from 1936 until his death in 1964. Robert Murray succeeded his late family on the Board of Trustees of the Manual. He chaired the Board from 1976 to 1990.

In addition to these responsibilities, Murray has served the microbiology community by his editorial guidance of various journals of the American Society for Microbiology and other international societies.

During his tenure at the University of Western Ontario, Murray and his colleagues and students conducted research that has greatly advanced the understanding of how **bacteria** are constructed and function. For example, the use of light and electron microscopy and techniques such as x-ray diffraction revealed the presence and some of the structural details of the so-called regularly structured (or RS) layer that overlays some bacteria. In another area, Murray discovered and revealed many structural and behavior aspects of a bacterium called *Deinococcus radiodurans*. This bacterium displays resistance to levels of radiation that are typically lethal to bacteria.

Such research has been acknowledged with a number of awards and honorary degrees. Murray's contribution to Canadian microbiology continues. He is a member of the Board of Directors of the Canadian Bacterial Diseases Network of Centres of Excellence.

See also Bacterial ultrastructure; Radiation resistant bacteria

MUTANTS: ENHANCED TOLERANCE OR SENSITIVITY TO TEMPERATURE AND pH RANGES

Microorganisms have optimal environmental conditions under which they grow best. Classification of microorganisms in terms of growth rate dependence on temperature includes the thermopiles, the mesophiles and psychrophiles. Similarly, while most organisms grow well in neutral **pH** conditions, some organisms grow well under acidic conditions, while others can grow under alkaline conditions. The mechanism by which such control exists is being studied in detail. This will overcome the need to obtain mutants by a slow and unsure process of acclimatization.

When some organisms are subjected to high temperatures, they respond by synthesizing a group of proteins that help to stabilize the internal cellular environment. These, called heat shock proteins, are present in both prokaryotes and **eukaryotes**. Heat stress specifically induces the **transcription** of genes encoding these proteins. Comparisons of amino acid sequences of these proteins from the **bacteria** ***Escherichia coli*** and the fruit fly *Drosophila* show that they are 40%–50% identical. This is remarkable considering the length of evolutionary time separating the two organisms.

Fungi are able to sense extracellular pH and alter the expression of genes. Some fungi secrete acids during growth making their environment particularly acidic. A strain of *Asperigillus nidulans* encodes a regulatory protein that activates transcription of genes during growth under alkaline conditions and prevents transcription of genes expressed in acidic conditions. A number of other genes originally found by analysis of mutants have been identified as mediating pH regulation, and some of these have been cloned. Improved understanding of pH sensing and regulation of **gene** expression will play an important role in gene manipulation for **biotechnology**.

The pH of the external growth medium has been shown to regulate gene expression in several enteric bacteria like *Vibrio cholerae*. Some of the acid-shock genes in *Salmonella* may turn out to assist its growth, possibly by preventing lysosomal acidification. Interestingly, acid also induces virulence in the plant pathogen (harmful microorganism) *Agrobacterium tumefaciens*.

Study of pH-regulated genes is slowly leading to knowledge about pH homeostasis, an important capability of many enteric bacteria by which they maintain intracellular pH. Furthermore, it is felt that pH interacts in important ways with other environmental and metabolic pathways involving anaerobiosis, sodium (Na^+) and potassium (K^+) levels, **DNA** repair, and amino acid degradation. Two different kinds of inducible pH homeostasis mechanisms that have been demonstrated are acid tolerance and the sodium-proton antiporter NhaA. Both cases are complex, involving several different stimuli and gene loci.

Salmonella typhimurium(the bacteria responsible for **typhoid fever**) that grows in moderately acid medium (pH 5.5–6.0) induces genes whose products enable cells to retain viability (ability to live) under more extreme acid conditions (below pH 4) where growth is not possible. Close to 100% of acid-tolerant (or acid-adapted) cells can recover from extreme-acid exposure and grow at neutral pH. The inducible survival mechanism is called acid tolerance response. The retention of viability by acid-tolerant cells correlates with improved pH homeostasis at low external pH represents inducible pH homeostasis.

Cells detect external alkalization with the help of a mechanism known as the alkaline signal **transduction** system. Under such environmental conditions, an inducible system for internal pH homeostasis works in *E. coli*. The so-called sodium-proton antiporter gene NhaA is induced at high external pH in the presence of high sodium. The NhaA antiporter acts to acidify the **cytoplasm** through proton/sodium exchange. This allows the microorganism to survive above its normal pH range. As *B. alkalophilus* may have as many as three sodium-proton antiporters, it is felt that the number of antiporters may relate to the alkalophilicity of a species.

The search for **extremophiles** has intensified recently. Standard **enzymes** stop working when exposed to heat or other extreme conditions, so manufacturers that rely on them must often take special steps to protect (stabilize) the proteins during reactions or storage. By remaining active when other enzymes would fail, enzymes from extremophiles (extremozymes) can potentially eliminate the need for those added steps, thereby increasing efficiency and reducing costs in many applications.

Many routes are being followed to use the capacity that such extremophiles possess. First, the direct use of these natural mutants to grow and produce the useful products. Also, it is possible with recombinant DNA technology to isolate genes from such organisms that grow under unusual conditions and clone them on to a fast growing organism. For example, an enzyme alpha-amylase is required to function at high temperature for the hydrolysis of starch to glucose. The gene for the enzyme was isolated from *Bacillus stearothermophilus,* an organism that is grows naturally at 194°F (90°C), and cloned into another suitable organism. Finally, attempts are being made to stabilize the proteins themselves by adding some groups (e.g., disulfide bonds) that prevent its easy denaturation. This process is called protein engineering.

Conventional mutagenesis and **selection** schemes can be used in an attempt to create and perpetuate a mutant form of a gene that encodes a protein with the desired properties. However, the number of mutant proteins that are possible after alteration of individual nucleotides within a structural gene by this method is extremely large. This type of mutagenesis also could lead to significant decrease in the activity of the enzyme. By using set techniques that specifically change amino-acids encoded by a cloned gene, proteins with properties that are better than those obtained from the naturally occurring strain can be obtained. Unfortunately, it is not possible to know in advance which particular amino acid or short sequence of amino acids will contribute to particular changes in physical, chemical, or kinetic properties. A particular property of a protein, for example, will be influenced by amino acids quite far apart in the linear chain as a consequence of the folding of the protein, which may bring them into close proximity. The amino acid sequences that would bring about change in physical properties of the protein can be obtained after characterization of the three dimensional structure of purified and crystallized protein using x-ray crystallography and other analytical procedures. Many approaches are being tried to bring about this type of "directed mutagenesis" once the specific nucleotide that needs to be altered is known.

See also Bacterial adaptation; Evolutionary origin of bacteria and viruses; Microbial genetics; Mutations and mutagenesis

MUTATIONS

A mutation is any change in genetic material that is passed on to the next generation. The process of acquiring change in genetic material forms the fundamental underpinning of **evolution**. Mutation is a source of genetic variation in all life forms. Depending on the organism or the source of the mutation, the genetic alteration may be an alteration in the organized collection of genetic material, or a change in the composition of an individual **gene**.

Mutations may have little impact, or they may produce a significant positive or negative impact, on the health, competitiveness, or function of an individual, family, or population.

Mutations arise in different ways. An alteration in the sequence, but not in the number of nucleotides in a gene is a nucleotide substitution. Two types of nucleotide substitution mutations are missense and nonsense mutations. Missense mutations are single base changes that result in the substitution of one amino acid for another in the protein product of the gene. Nonsense mutations are also single base changes, but create a termination codon that stops the **transcription** of the gene. The result is a shortened, dysfunctional protein product.

Another mutation involves the alteration in the number of bases in a gene. This is an insertion or deletion mutation. The impact of an insertion or deletion is a frameshift, in which the normal sequence with which the genetic material is interpreted is altered. The alteration causes the gene to code for a different sequence of amino acids in the protein product than would normally be produced. The result is a protein that functions differently—or not all—as compared to the normally encoded version.

Genomes naturally contain areas in which a nucleotide repeats in a triplet. Trinucleotide repeat mutations, an increased number of triplets, are now known to be the cause of at least eight genetic disorders affecting the nervous or neuromuscular systems.

Mutations arise from a number of processes collectively termed mutagenesis. Frameshift mutations, specifically insertions, result from mutagenic events where **DNA** is inserted into the normally functioning gene. The genetic technique of insertional mutagenesis relies upon this behavior to locate target genes, to study gene expression, and to study protein structure-function relationships.

DNA mutagenesis also occurs because of breakage or base modification due to the application of radiation, chemicals, ultraviolet light, and random replication errors. Such mutagenic events occur frequently, and the cell has evolved repair mechanisms to deal with them. High exposure to DNA damaging agents, however, can overwhelm the repair machinery.

Genetic research relies upon the ability to induce mutations in the lab. Using purified DNA of a known restriction map, site-specific mutagenesis can be performed in a number of ways. Some **restriction enzymes** produce staggered nicks at the site of action in the target DNA. Short pieces of DNA

(linkers) can subsequently be introduced at the staggered cut site, to alter the sequence of the DNA following its repair. Cassette mutagenesis can be used to introduce selectable genes at the specific site in the DNA. Typically, these are drug-resistance genes. The activity of the insert can then be monitored by the development of resistance in the transformed cell. In deletion formation, DNA can be cut at more than one restriction site and the cut regions can be induced to join, eliminating the region of intervening DNA. Thus, deletions of defined length and sequence can be created, generating tailor-made deletions. With site-directed mutagenesis, DNA of known sequence that differs from the target sequence of the original DNA, can be chemically synthesized and introduced at the target site. The insertion causes the production of a mutation of pre-determined sequence. Site-directed mutagenesis is an especially useful research tool in inducing changes in the shape of proteins, permitting precise structure-function relationships to be probed. Localized mutagenesis, also known as heavy mutagenesis, induces mutations in a small portion of DNA. In many cases, mutations are identified by the classical technique of phenotypic identification—looking for an alteration in appearance or behavior of the mutant.

Mutagenesis is exploited in **biotechnology** to create new **enzymes** with new specificity. Simple mutations will likely not have as drastic an effect as the simultaneous alteration of multiple amino acids. The combination of mutations that produce the desired three-dimensional change, and so change in enzyme specificity, is difficult to predict. The best progress is often made by creating all the different mutational combinations of DNA using different **plasmids**, and then using these **plasmids** as a mixture to transform ***Escherichia coli* bacteria**. The expression of the different proteins can be monitored and the desired protein resolved and used for further manipulations.

See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Chemical mutagenesis; Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); Laboratory techniques in immunology; Mitochondrial DNA; Mitochondrial inheritance; Molecular biology and molecular genetics

MYCELIUM

Mycelium (plural, mycelia) is an extension of the **hyphae** of **fungi**. A hyphae is a thread-like, branching structure formed by fungi. As the hyphae grows, it becomes longer and branches off, forming a mycelium network visually reminiscent of the branches of tree.

The mycelium is the most important and permanent part of a fungus. The mycelia network that emanates from a fungal spore can extend over and into the soil in search of nutrients. The ends of some mycelia terminate as mushrooms and toadstools.

Mycelium have been recognized as fungal structures for a long time. The author Beatrix Potter provided accurate sketches of mycelium over 100 years ago. At the time her observations were considered irrelevant and the significance of mycelium was lost until some years after her work.

The growth of mycelia can be extensive. A form of honey fungus found in the forests of Michigan, which began from a single spore and grows mainly underground, now is estimated to cover 40 acres. The mycelia network is thought to be over 100 tons in weight and is at least 1,500 years old. More recently, another species of fungus discovered in Washington State was found to cover at least 1,500 acres

The initial hyphae produced by a fungus has only one copy of each of its **chromosomes**. Thus, it is haploid. The resulting mycelium will also be haploid. When one haploid mycelium meets another haploid mycelium of the same species, the two mycelia can fuse. The fused cells then contain two nuclei. In contrast to plants and animals, where the nuclei would fuse, forming a functional **nucleus** containing two copies of each chromosome (a diploid state), the two nuclei in the fugal cell remain autonomous and function separate from one another.

Fusion of the nuclei does occur as a prelude to spore formation. Several duplications and shuffling of the genetic material produces four spores, each with a unique genetic identity.

At any one time, part of a mycelia network may be actively growing while another region may be dormant, awaiting more suitable conditions for growth. Mycelium is able to seek out such suitable conditions by moving towards a particular food source, such as a root. Also mycelium can change their texture, for example from a fluffy state to a thin compressed state or to thicker cord-like growths. All these attributes enable the mycelium to ensure the continued growth of the fungus.

See also Armillaria ostoyae; Fungal genetics

MYCOBACTERIAL INFECTIONS, ATYPICAL

Atypical mycobacteria are species of mycobacteria that are similar to the mycobacteria that are the cause of **tuberculosis**. Like other mycobacteria, they are rod-like in shape and they are stained for observation by light microscopy using a specialized staining method called acid-fast staining. The need for this staining method reflects the unusual cell wall chemistry of mycobacteria, relative to other **bacteria**. In contrast to other mycobacteria, atypical mycobacteria do not cause tuberculosis. Accordingly, the group of bacteria is also described as nonpneumoniae mycobacteria. This group of bacteria is also designated as MOTT (mycobacteria other than tuberculosis). Examples of atypical mycobacteria include *Mycobacterium kansasii*, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium marinum*, and *Mycobacterium ulcerans*.

The atypical mycobacteria are widely present in the environment. They inhabit fresh and salt water, milk, soil, and the feces of birds. Other environmental niches, which so far have not been determined, are possible. The nature of their habitats suggests that transmission to people via soiled or dirty hands, and the ingestion of contaminated water or milk would

be typical. Yet, little is still known about how people become contaminated. One species, known as *Mycobacterium marinum*, is found in swimming pool water, and can cause a skin infection in fingers or toes upon contact with the skin of a swimmer. Additionally, some evidence supports the transmission of atypical mycobacteria in aerosols (that is, as part of tiny droplets that can drift through the air and become inhaled).

Contamination with atypical mycobacteria may be a natural part of life. For the majority of people, whose immune systems are functioning efficiently, the microbe does not establish an infection. However, for those who **immune system** is not operating well, the presence of the atypical mycobacteria is a problem. Indeed, for those afflicted with acquired **immunodeficiency** syndrome (**AIDS**), infection with atypical mycobacteria (typically with *Mycobacterium avium* and *Mycobacterium intracellulare*) is almost universal.

Atypical mycobacteria tend to first establish a foothold in the lungs. From there the bacteria can spread, via the bloodstream, throughout the body. Infections in almost every organ of the body can ensue. Examples of sites of infection include the brain, lymph nodes, spleen, liver, bone marrow, and gastrointestinal tract. The overwhelming nature of the infections can be fatal, especially to people already weakened by AIDS.

The spectrum of infection sites produces a wide range of symptoms, which include a feeling of malaise, nausea, worsening diarrhea, and, if the brain is affected, headaches, blurred vision, and loss of balance.

Infrequently, those with healthy immune systems can acquire an atypical mycobacterial infection. The result can be a bone infection (osteomyelitis), a form of arthritis known as septic arthritis, and localized infections known as abscesses.

The diagnosis of infection caused by atypical mycobacteria is complicated by the fact that the growth of the **microorganisms** on conventional laboratory **agar** is very difficult. Specialized growth medium is required, which may not be available or in stock in every clinical laboratory. The delay in diagnosis can result in the explosive development of multiorgan infections that are extremely difficult to treat.

Treatment of atypical mycobacteria is complicated by the unusual cell wall possessed by the bacterium, relative to other bacteria. The cell wall is made predominantly of lipids. Partially as a result of their wall construction, atypical mycobacteria are not particularly susceptible to antibiotic therapy. As well, aggressive therapy is often not possible, given the physical state of the AIDS patient being treated. A prudent strategy for AIDS is the use of certain drugs as a means of preventing infection, and to try to avoid those factors that place the individual at risk for acquiring atypical mycobacterial infections. Some risk factors that have been identified include the avoidance of unwashed raw fruit and vegetables. As well, contact with pigeons should be limited, since these birds are known to harbor atypical mycobacteria in their intestinal tracts.

See also Bacteria and bacterial infections; Immunodeficiency diseases

MYCOLOGY

Mycology is the study of **fungi**, including molds and yeasts. The study of mycology encompasses a huge number of **microorganisms**. Indeed, just considering molds, the estimates of the number of species ranges from the tens of thousands to over 300,000.

Fungi are eukaryotic microorganisms (**eukaryotes** have their nucleic material contained within a membrane), which can produce new daughter fungi by a process similar to **bacteria**, where the nuclear material replicates and then the cell splits to form two daughter cells, or via sexual reproduction, where nuclear material from two fungi are mixed together and the daughter cells inherit material from both parents. Growth of fungi can occur either by the budding off of the new daughter cells from the parent or by the extension of the branch (or **hyphae**) of a fungus.

The study of fungi can take varied forms. Discovery of new fungi and their grouping with the existing fungi is one aspect of mycology. Unraveling the chemical nature of the fungal survival and growth is another aspect of mycology. For example, some fungi produce **antibiotics** such as **penicillin** as part of their defensive strategies. This aspect of mycology has proved to be extremely important for human health. The adverse effects of fungi on human health and plants constitutes yet another aspect of mycology. Still another aspect of mycology, which can encompass some of the preceding, is concerned with the economic impact, beneficial or not, of fungi. For example, those fungi that are edible or which produce antibiotics have a tremendous positive economic impact, whereas fungi that cause damage to agricultural plants exact a negative economic toll.

Some mycologists (scientists who study fungi) conduct extensive research into the origin of fungi. The discovery of fossilized fungi that resemble those from the four major groups of modern fungi in rocks that date back 360–410 million years indicate that fungi were already well-established and diversifying even before other forms of life had made the transition from the sea to the land.

Mycology has resulted in the classification of fungi into four divisions. These divisions are the Chytridiomycota, Zygomycota (which include the bread molds such as Neurospora), Ascomycota (which include yeasts), and the Basidiomycota. **Lichens** do not fit this classification, as lichens are not single-celled fungi. Rather, they are a symbiotic association (an association that is beneficial for both participants) between a fungus and an alga.

The health-oriented aspect of mycology is important, particularly as the danger of fungal infections, especially to those whose **immune system** is compromised, has been recognized since the identification of acquired **immunodeficiency** syndrome in the 1970s.

For example, in those whose immune systems are functioning properly, an infection with the **mold** known as *Aspergillus* can produce a mild allergic type of reaction. However, in those people whose immune systems are not operating efficiently, the mold can grow in the lungs, and can produce a serious infection called bronchopulmonary

aspergillosis. As well, a more invasive infection via the bloodstream can result in mold growth in the eye, heart, kidneys, and the skin. The invasive infection can be lethal.

Mycologists are becoming increasingly involved in the remediation of buildings. The so-called "sick building syndrome" is often due to the growth of fungi, particularly molds, in the insulation of buildings. The growth of the molds including *Cladosporium, Penicilium, Alternaria, Aspergillus,* and *Mucor* can produce allergic reactions ranging from inconvenient to debilitating to building users.

See also Candidiasis; Economic uses and benefits of microorganisms; Slime molds

MYCOPLASMA INFECTIONS

Mycoplasma are **bacteria** that lack a conventional cell wall. They are capable of replication. Mycoplasma cause various diseases in humans, animals, and plants.

There are seven species of mycoplasma that are known to cause disease in humans. *Mycoplasma pneumoniae* is an important cause of sore throat, **pneumonia**, and the **inflammation** of the channels in the lung that are known as the bronchi. Because of the atypical nature of the bacterium, mycoplasma-induced pneumonia is also referred to as atypical pneumonia. The pneumonia can affect children and adults. The symptoms tend to be more pronounced in adults. In fact, children may not exhibit any symptoms of infection. Symptoms of infection include a fever, general feeling of being unwell, sore throat, and sometimes an uncomfortable chest. These symptoms last a week to several months and usually fade without medical intervention.

Mycoplasma pneumoniae can also cause infections in areas of the body other than the lungs, including the central nervous system, liver, and the pancreas.

Another species, *Mycoplasma genitalium*, is associated with infections of the urethra, especially when the urethra has been infected by some other bacteria. The mycoplasma infection may occur due to the stress imposed on the **immune system** by the other infection.

A mycoplasma called *Ureaplasma urealyticum* is present in the genital tract of many sexually active women. The resulting chronic infection can contribute to premature delivery in pregnant women. As well, the mycoplasma can be transmitted from the mother to the infant. The infant can contract pneumonia, infection of the central nervous system, and lung malfunction.

A group of four mycoplasma species are considered to be human pathogens and may contribute to the development an **immunodeficiency** virus infection to the more problematic and debilitating symptoms of Acquired Immunodeficiency Syndrome (**AIDS**). The species of mycoplasma are *Mycoplasma fermentans*, *Mycoplasma pirum*, *Mycoplasma hominis*, and *Mycoplasma penetrans*.

Mycoplasma have also been observed in patients who exhibit other diseases. For example, studies using genetic probes and the **polymerase chain reaction** technique of detecting target **DNA** have found *Mycoplasma fermentans* in upwards of 35% of those afflicted with chronic fatigue syndrome. The bacterium is present in less than 5% of healthy populations. Similar percentages have been found in soldiers of the Persian Gulf War who are exhibiting chronic fatigue-like symptoms. While the exact relationship between mycoplasma and the chronic fatigue state is not fully clear, the current consensus is that the bacteria is playing a secondary role in the development of the symptoms.

Over 20 years ago, mycoplasma was suggested as a cause of rheumatoid arthritis. With the development of molecular techniques of bacterial detection, this suggestion could be tested. The polymerase chain reaction has indeed detected *Mycoplasma fermentans* in a significant number of those afflicted with the condition. But again, a direct causal relationship remains to be established.

The association of mycoplasma with diseases like arthritis and chronic fatigue syndrome, which has been implicated with a response of the body's immune system against its own components, is consistent with the growth and behavior of mycoplasma. The absence of a conventional cell wall allows mycobacteria to penetrate into the white blood cells of the immune system. Because some mycoplasma will exist free of the blood cells and because the bacteria are capable of slow growth in the body, the immune system will detect and respond to a mycobacterial infection. But this response is generally futile. The bacteria hidden inside the white blood cells will not be killed. The immune components instead might begin to attack other antigens of the host that are similar in three-dimensional structure to the mycobacterial antigens. Because mycoplasma infections can become chronic, damage to the body over an extended time and the stress produced on the immune system may allow other **microorganisms** to establish infections.

The polymerase chain reaction is presently the best means of detecting mycoplasma. The bacteria cannot be easily grown on laboratory media. Labs that test using the polymerase technique are still rare. Thus, a mycoplasma infection might escape detection for years.

Strategies to eliminate mycoplasma infections are now centering on the strengthening of the immune system, and long-term antibiotic use (e.g., months or years). Even so, it is still unclear whether **antibiotics** are truly effective on mycoplasma bacteria. Mycoplasma can alter the chemical composition of the surface each time a bacterium divides. Thus, there may be no constant target for an antibiotic.

See also Bacteria and bacterial infection; Bacterial membranes and cell wall

N

VAN NEIL, CORNELIUS B. (1897-1985)

Dutch microbiologist and teacher

Cornelius B. van Neil made pioneering contributions to the study of **photosynthesis** in the **bacteria** that are known as the purple and green sulfur bacteria. These rather exotic bacteria are plant-like in that they use specific wavelengths of sunlight as a source of energy, instead of the **metabolism** of carbon-containing compounds. In addition to his research contributions, van Neil is noteworthy because of his tremendous teaching contributions. He inspired many people to take up a career in research microbiology in the first half of the twentieth century. Several of his students went on to obtain the Nobel Prize for their scientific contributions.

Van Neil was born in Haarlem, The Netherlands. His interest in chemistry was sparked while he was still in high school. This interest led him to enroll in the Chemistry Division of the Technical University of The Netherlands. His education was interrupted by a brief stint in the Dutch army. But ultimately he received a degree in Chemical Engineering in 1923. He then became a laboratory assistant to **Albert Jan Kluyver**, a renowned microbial physiologist and taxonomist. van Neil was responsible for the **culture** collection of **yeast**, bacteria, and **fungi** that Kluyver has amassed. During this time, van Neil isolated *Chromatium spp*. and *Thiosarcina rosea* and demonstrated that their growth did not involve the production of oxygen.

van Neil received a Ph.D. from The Technical University in 1928 for his research on proprionic acid bacteria (now well-known as one of the causes of acne). Following this, he came to the United States to accept a position at the Hopkins Marine Station, a research institution of Stanford University located on the Monterey Peninsula. He remained at Hopkins until his retirement in 1962. From 1964 until 1968, he was a visiting Professor at the University of California at Santa Cruz. He then retired from teaching and research entirely.

During his tenure at the Hopkins Marine Station, van Neil produced his most fundamentally important work. He was able to demonstrate that the ability of purple and green sulfur bacteria to exist without oxygen depends on the presence of sunlight. The photosynthetic reaction causes carbon dioxide to become reduced, providing the building blocks needed by the bacteria for growth and division. van Neil went on to broaden his work to photosynthesis in general. His observations that radiant energy activates a hydrogen donating compound instead of carbon dioxide was seminal in the development of subsequent studies of photosynthetic reactions in nature.

Another area where van Neil made a fundamental contribution was the emerging field of bacterial classification. Through his efforts in identifying over 150 strains of bacteria, and consolidating these organisms into six species contained within the two genera of *Rhodopseudomonas* and *Rhodo-spirillum*, van Neil and Kluyver laid the groundwork for the use of bacterial physical and chemical characteristics as a means of classifying bacteria.

van Neil's teaching legacy is as important as his research contributions. He established the first course in general microbiology in the United States. He was a riveting lecturer, and his classes could last an entire day. He taught and mentored many students who went on to considerable achievements of their own.

See also Microbial taxonomy; Photosynthetic microorganisms

NEISSERIA • *see* GONORRHEA

NEOMYCIN • *see* ANTIBIOTICS

NEURAMINIDASE (NA) • *see* HEMAGGLUTININ (HA) AND NEURAMINIDASE (NA)

NEUROSPORA

The bread **mold** *Neurospora crassa* is a simple fungal eukaryote which has been used extensively as a model organism to

elucidate many of the principles of genetics of higher organisms. It is relatively easy to cultivate in the laboratory. *Neurospora* are eukaryotic organisms; that is, they organize their genes onto **chromosomes**. They may exist as either diploid cells (two copies of **gene** and chromosome) or haploid (one copy of each gene and chromosome). *Neurospora* has both a sexual and an asexual reproductive cycle which allows exploration of genetic processes more complex than those found in **bacteria**.

The asexual cycle consists of a filamentous growth of haploid mycelia. This stage is the vegetative stage. While the nuclei in this stage are indeed haploid, the tubular filaments contain multiple nuclei often without the distinction of individual cells. Under conditions of sparse food resources, the filaments (called **hyphae**) become segmented producing bright orange colored macroconidia, asexual spores that can become detached and are more readily dispersed throughout the environment. Asexual spores can develop again into multicellular hyphae, completing the cycle. Asexual spores can also function as male gametes in the sexual reproductive cycle.

The sexual part of the life cycle begins with the mature fruiting body called the perithecium. These are sacs of sexual spores (ascospores) resulting from meiotic division. The sexual spores are discharged from the perithecium and can germinate into haploid cultures or fuse with conidia of complementary mating types. There are two genetically distinct mating types A and a. *Neurospora* cannot self fertilize, rather haploid sexual spores of opposite mating types must be joined at fertilization. Nuclear fusion of the male and female gametes occurs setting the stage for meiotic division to form ascospores. The diploid stage is brief as nuclear fusion quickly gives way to two meiotic divisions that produce eight ascospores. Ascospores are normally black and shaped like a football. The physical position of the ascospores is linear and corresponds to the physical position of the individual chromosomes during meiosis. In the absence of crossing over, the four a-mating type ascospores are next to each other followed by the four A-mating type ascospores.

The existence of a large collection of distinct mutant strains of *Neurospora* and the linear array of the products of meiosis makes *Neurospora* an ideal organism for studying mutation, chromosomal rearrangements, and **recombination**. As a relatively simple eukaryote, *Neurospora* has permitted study of the interactions of nuclear genes with mitochondrial genes. *Neurospora* also exhibits a normal circadian rhythm in response to light in the environment, and much of the fundamental genetics and biology of circadian clock cycles (chronobiology) have been elucidated through the careful study of mutant cells which exhibit altered circadian cycles.

See also Microbial genetics

NITROGEN CYCLE IN MICROORGANISMS

Nitrogen is a critically important nutrient for organisms, including **microorganisms**. This element is one of the most abundant elemental constituents of eukaryotic tissues and prokaryotic cell walls, and is an integral component of amino acids, proteins, and nucleic acids.

Most plants obtain their nitrogen by assimilating it from their environment, mostly as nitrate or ammonium dissolved in soil water that is taken up by roots, or as gaseous nitrogen oxides that are taken up by plant leaves from the atmosphere. However, some plants live in a symbiotic relationship with microorganisms that have the ability to fix atmospheric nitrogen (which can also be called dinitrogen) into ammonia. Such plants benefit from access to an increased supply of nitrogen.

As well, nitrogen-assimilating microorganisms are of benefit to animals. Typically animals obtain their needed nitrogen through the plants they ingest. The plant's organic forms of nitrogen are metabolized and used by the animal as building blocks for their own necessary biochemicals. However, some animals are able to utilize inorganic sources of nitrogen. For example, ruminants, such as the cow, can utilize urea or ammonia as a consequence of the metabolic action of the microorganisms that reside in their forestomachs. These microbes can assimilate nitrogen and urea and use them to synthesize the amino acids and proteins, which are subsequently utilized by the cow.

Nitrogen (N) can occur in many organic and inorganic forms in the environment. Organic nitrogen encompasses a diversity of nitrogen-containing organic molecules, ranging from simple amino acids, proteins, and nucleic acids to large and complex molecules such as the humic substances that are found in soil and water.

In the atmosphere, nitrogen exists as a diatomic gas (N_2). The strong bond between the two nitrogen atoms of this gas make the molecule nonreactive. Almost 80% of the volume of Earth's atmosphere consists of diatomic nitrogen, but because of its almost inert character, few organisms can directly use this gas in their nutrition. Diatomic nitrogen must be "fixed" into other forms by certain microorganisms before it can be assimilated by most organisms.

Another form of nitrogen is called nitrate (chemically displayed as NO_3-). Nitrate is a negatively charged ion (or anion), and as such is highly soluble in water.

Ammonia (NH_{3S}) usually occurs as a gas, vapor, or liquid. Addition of a hydrogen atom produces ammonium (NH_4+). Like nitrate, ammonium is soluble in water. Ammonium is also electrochemically attracted to negatively charged surfaces associated with clays and organic matter in soil, and is therefore not as mobile as nitrate.

These, and the other forms of nitrogen are capable of being transformed in what is known as the nitrogen cycle.

Nitrogen is both very abundant in the atmosphere and is relatively inert and nonreactive. To be of use to plants, dinitrogen must be "fixed" into inorganic forms that can be taken up by roots or leaves. While dinitrogen fixation can occur non-biologically, biological fixation of dinitrogen is more prevalent.

A bacterial enzyme called nitrogenase is capable of breaking the tenacious bond that holds the two nitrogen atoms together. Examples of nitrogen-fixing **bacteria** include *Azotobacter*, *Beijerinkia*, some species of *Klebsiella*, *Clostridium*, *Desulfovibrio*, purple sulfur bacteria, purple non-sulfur bacteria, and green sulfur bacteria.

Some species of plants live in an intimate and mutually beneficial symbiosis with microbes that have the capability of fixing dinitrogen. The plants benefit from the symbiosis by having access to a dependable source of fixed nitrogen, while the microorganisms benefit from energy and habitat provided by the plant. The best known symbioses involve many species in the legume family (Fabaceae) and strains of a bacterium known as *Rhizobium japonicum*. Some plants in other families also have dinitrogen-fixing symbioses, for example, red alder (*Alnus rubra*) and certain member of Actinomycetes. Bacteria from the genera *Frankia* and *Azospirillum* are also able to establish symbiotic relationships with non-leguminous plants. Many species of **lichens**, which consist of a symbiotic relationship between a fungus and a blue-green bacterium, can also fix dinitrogen.

Ammonification is a term for the process by which the organically bound nitrogen of microbial, plant, and animal biomass is recycled after their death. Ammonification is carried out by a diverse array of microorganisms that perform ecological decay services, and its product is ammonia or ammonium ion. Ammonium is a suitable source of nutrition for many species of plants, especially those living in acidic soils. However, most plants cannot utilize ammonium effectively, and they require nitrate as their essential source of nitrogen nutrition.

Nitrate is synthesized from ammonium by an important bacterial process known as nitrification. The first step in nitrification is the oxidation of ammonium to nitrite (NO_2-), a function carried out by bacteria in the genus *Nitrosomonas*. Once formed, the nitrite is rapidly oxidized further to nitrate, by bacteria in the genus *Nitrobacter*. The bacteria responsible for nitrification are very sensitive to acidity, so this process does not occur at significant rates in acidic soil or water.

Denitrification is another bacterial process, carried out by a relatively wide range of species. In denitrification, nitrate is reduced to either nitrous oxide or dinitrogen, which is then emitted to the atmosphere. One of the best studies bacterial examples is *Pseudomonas stutzeri*. This bacterial species has almost 50 genes that are known to have a direct role in denitrification. The process of denitrification occurs under conditions where oxygen is not present, and its rate is largest when concentrations of nitrate are large. Consequently, fertilized agricultural fields that are wet or flooded can have quite large rates of denitrification. In some respects, denitrification can be considered to be an opposite process to dinitrogen fixation. In fact, the global rates of dinitrogen fixation and denitrification are in an approximate balance, meaning that the total quantity of fixed nitrogen in Earth's ecosystems is neither increasing nor decreasing substantially over time.

See also Biogeochemical cycles; Economic uses and benefits of microorganisms

NON-CULTURABLE BACTERIA • *see* VIABLE BUT NON-CULTURABLE BACTERIA

NON-SELECTIVE MEDIA • *see* GROWTH AND GROWTH MEDIA

NON-SPECIFIC IMMUNITY • *see* IMMUNITY, ACTIVE, PASSIVE, AND DELAYED

NOSOCOMIAL INFECTIONS

A nosocomial infection is an infection that is acquired in a hospital. More precisely, the **Centers for Disease Control** in Atlanta, Georgia, defines a nosocomial infection as a localized infection or one that is widely spread throughout the body that results from an adverse reaction to an infectious microorganism or toxin that was not present at the time of admission to the hospital.

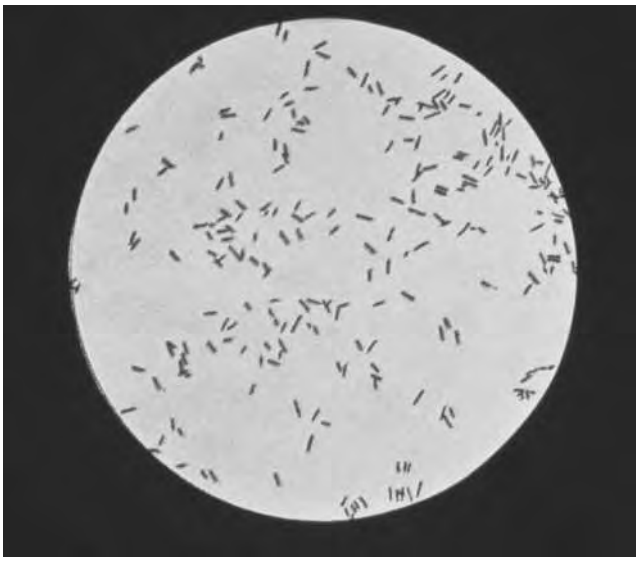
The term nosocomial infection derives from the *nosos*, which is the Greek word for disease.

Nosocomial infections have been a part of hospital care as long as there have been hospitals. The connection between the high death rate of hospitalized patients and the exposure of patients to infectious **microorganisms** was first made in the mid-nineteenth century. Hungarian physician **Ignaz Semmelweis** (1818–1865) noted the high rate of death from puerperal fever in women who delivered babies at the Vienna General Hospital. Moreover, the high death rate was confined to a ward at which medical residents were present. Another ward, staffed only by midwives who did not interact with other areas of the hospital, had a much lower death rate. When the residents were made to wash their hands in a disinfectant solution prior to entering the ward, the death rate declined dramatically.

At about the same time, the British surgeon **Joseph Lister** (1827–1912) also recognized the importance of hygienic conditions in the operating theatre. His use of phenolic solutions as sprays over surgical wounds helped lessen the spread of microorganisms resident in the hospital to the patient. Lister also required surgeons to wear rubber gloves and freshly laundered operating gowns for surgery. He recognized that infections could be transferred from the surgeon to the patient. Lister's actions spurred a series of steps over the next century, which has culminated in today's observance of sterile or near-sterile conditions in the operating theatre.

Despite these improvements in hospital hygienic practices, the chance of acquiring a nosocomial infection still approaches about 10%. Certain hospital situations are even riskier. For example, the chance of acquiring a urinary tract infection increases by 10% for each day a patient is equipped with a urinary catheter. The catheter provides a ready route for the movement of **bacteria** from the outside environment to the urinary tract.

The most common microbiological cause of nosocomial infection is bacteria. The microbes often include both Gram-negative and Gram-positive bacteria. Of the Gram-negative bacteria, *Escherichia coli*, *Proteus mirabilis,* and other members of the family known as Enterobacteriacaea are predominant. These bacteria are residents of the intestinal tract. They are spread via fecal **contamination** of people, instruments or



***Pseudomonas aeruginosa*, an important cause of nosocomial infections.**

other surfaces. Other Gram-negative bacteria of consequence include members of the genera *Pseudomonas* and *Acinetobacter*.

Gram-positive bacteria, especially *Staphylococcus aureus*, frequently cause infections of wounds. This bacterium is part of the normal flora on the surface of the skin, and so can readily gain access to a wound or surgical incision.

One obvious cause of nosocomial infections is the state of the people who require the services of a hospital. Often people are ill with ailments that adversely affect the ability of their immune systems to recognize or combat infections. These people are more vulnerable to disease than they would otherwise be. A hospital is a place where, by its nature, infectious microorganisms are encountered more often than in other environments, such as the home or workplace. Simply by being in a hospital, a person is exposed to potentially disease-causing microorganisms.

A compounding factor, and one that is the cause of many nosocomial infections, is the developing resistance of bacteria to a number of **antibiotics** in common use in hospitals. For example, strains of *Staphylococcus aureus* that are resistant to all but a few conventional antibiotics are encountered in hospitals so frequently as to be almost routine. Indeed, many hospitals now have contingency plans to deal with outbreaks of these infections, which involve the isolation of patients, **disinfection** of affected wards, and monitoring of other areas of the hospital for the bacteria. As another example, a type of bacteria known as enterococci has developed resistance to virtually all antibiotics available. Ominously, the genetic determinant for the multiple **antibiotic resistance** in enterococci has been transferred to *Staphylococcus aureus* in the laboratory setting. Were such genetic transfer to occur in the hospital setting, conventional antibiotic therapy for *Staphylococcus aureus* infections would become virtually impossible.

See also Bacteria and bacterial infection; History of public health; History of the development of antibiotics

NOTOBIOTIC ANIMALS • *see* ANIMAL MODELS OF INFECTION

NUCLEUS

The nucleus is a membrane-bounded organelle found in eukaryotic cells that contains the **chromosomes** and nucleolus. Intact eukaryotic cells are comprised of a nucleus and **cytoplasm**. A nuclear envelope encloses chromatin, the nucleolus, and a matrix which fills the nuclear space.

The chromatin consists primarily of the genetic material, **DNA**, and histone proteins. Chromatin is often arranged in fiber like nucleofilaments.

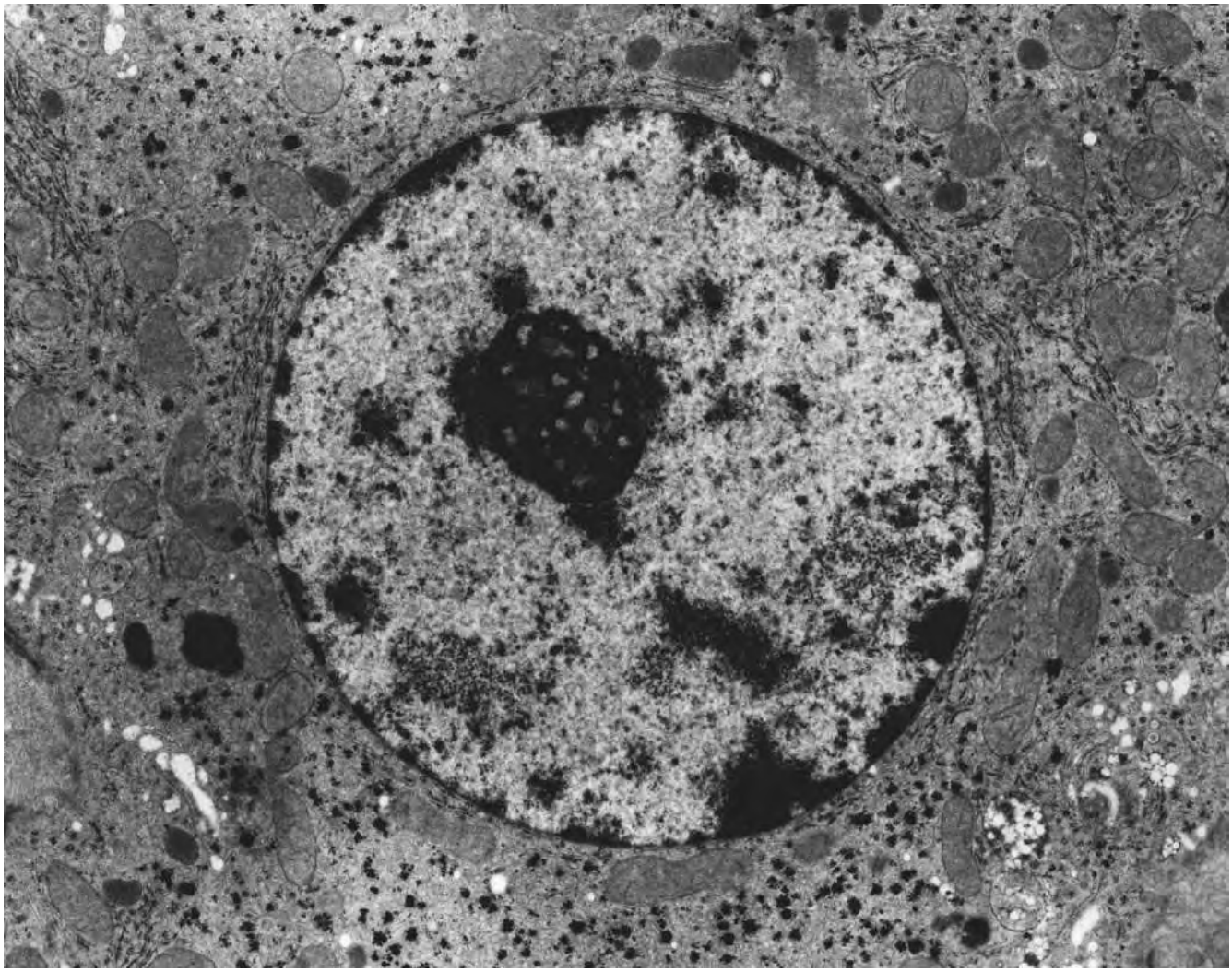
The nucleolus is a globular cell organelle important to ribosome function and **protein synthesis**. The nucleolus is a small structure within the nucleus that is rich in ribosomal **RNA** and proteins. The nucleolus disappears and reorganizes during specific phases of cell division. A nucleus may contain from one to several nucleoli. Nucleoli are associated with protein synthesis and enlarged nucleoli are observed in rapidly growing embryonic tissue (other than cleavage nuclei), cells actively engaged in protein synthesis, and malignant cells. The nuclear matrix itself is also protein rich.

The genetic instructions for an organism are encoded in nuclear DNA that is organized into chromosomes. Eukaryotic chromosomes are composed of proteins and nucleic acids (nucleoprotein). Accordingly, cell division and reproduction require a process by which the DNA (or in some prokaryotes, RNA) can be duplicated and passed to the next generation of cells (daughter cells)

It is possible to obtain genetic replicates through process termed nuclear transplantation. Genetic replicas are cloned by nuclear transplantation. The first **cloning** program using nuclear transplantation was able, as early a 1952, to produce frogs by nuclear transplantation. Since that time, research programs have produced an number of different species that can be cloned. More recently, sheep (Dolly) and other creatures have been produced by cloning nuclei from adult animal donors.

The cloning procedures for frogs or mammals are similar. Both procedures require the insertion of a nucleus into an egg that has been deprived of its own genetic material. The reconstituted egg, with a new nucleus, develops in accordance with the genetic instructions of the nuclear donor.

There are, of course, cells which do not contain the usual nuclear structures. Embryonic cleavage nuclei (cells forming a blastula) do not have a nucleolus. Because the cells retain the genetic competence to produce nucleoli, gastrula and all later cells contain nucleoli. Another example is found upon examination of mature red blood cells, erythrocytes, that in most mammals are without (devoid) of nuclei. The loss of nuclear material, however, does not preclude the competence to carry oxygen.



Thin section electron micrograph of a nucleus from a eukaryotic cell, showing the membrane that surrounds the nuclear contents.

See also Cell cycle (eukaryotic), genetic regulation of; DNA (deoxyribonucleic acid)

NUTTALL, GEORGE H. F. (1862-1937)

American bacteriologist

George Henry Falkiner Nuttall is noteworthy for his accomplishments while at Cambridge University in England; his research was concerned with **parasites** and of insect carriers of microbiological diseases. He was instrumental in establishing a diploma course in tropical medicine. In 1907, Nuttall moved into a new laboratory. There, he carried out research that clarified the disease of piroplasmosis, a still-serious disease of domesticated animals such as cattle. He showed that Trypan Blue could be used as a treatment. During this period, space limitations of the laboratory prompted him to seek funding to build and equip a new institute for parasitological research.

His efforts were successful, and he established the Molteno Institute for Research in Parasitology at Cambridge in 1921. The institute was named in honor of a South African farming family who were the principle financial backers of the initiative. He became the institute's first Director.

Nuttall's years at The Molteno Institute were spent in parasitological research and research on the cytochrome system of insects.

Nuttall was born in San Francisco. His early years were spent in Europe. He returned to America to train as a physician, receiving his M.D. from the University of California in 1884. He then undertook research on various microbiological and immunological projects in laboratories in North America and Europe. His burgeoning interest in parasitology and the role of insects and other agents of disease transmission led him to pursue further study. He received a Ph.D. in biology from the University of Göttingen in 1890. In 1899, at the age of 38, he moved to Cambridge, where he became a full Professor of biology in 1906.

Nuttall also contributed much the scientific literature. In 1901, he established and was founding editor of the *Journal of Hygiene*, and in 1908 founded and edited *Parasitology*. His writing includes *Blood Immunity and Blood Relationships* in 1904 and *The Bacteriology of Diphtheria* in 1908.

See also Parasites; Transmission of pathogens

O

O157:H7 INFECTION • *see* E. COLI O157:H7 INFECTION

ONCOGENE

An oncogene is a special type of **gene** that is capable of transforming host cells and triggering carcinogenesis. The name is derived from the Greek *onkos*, meaning bulk, or mass, because of the ability to cause tumor growth. Oncogenes were first discovered in **retroviruses** (**viruses** containing the enzyme reverse transcriptase, and **RNA**, rather than **DNA**) that were found to cause cancer in many animals (e.g., feline leukemia virus, simian sarcoma virus). Although this is a relatively common mechanism of oncogenesis in animals, very few oncogene-carrying viruses have been identified in man. The ones that are known include the papilloma virus HPV16 that is associated with cervical cancer, HTLV-1 and HTLV-2 associated with T-cell leukemia, and HIV-1 associated with Kaposi sarcoma.

Studies of humans led to the discovery of related genes called proto-oncogenes that exist naturally in the human genome. These genes have DNA sequences that are similar to oncogenes, but under normal conditions, the proto-oncogenes do not cause cancer. However, specific **mutations** in these genes can transform them to an oncogenic form that may lead to carcinogenesis. So, in humans, there are two unique ways in which oncogenesis occurs, by true viral infection and by mutation of proto-oncogenes that already exist in human cells.

See also Molecular biology and molecular genetics; Oncogenetic research; Viral genetics; Viral vectors in gene therapy; Virology; Virus replication; Viruses and responses to viral infection

ONCOGENE RESEARCH

Research into the structure and function of oncogenes has been a major endeavor for many years. The first chromosome rearrangement (Ph') involving a proto-oncogene to be directly associated with cancer induction was identified in 1960. Since then, over 50 proto-oncogenes have been mapped in the human genome, and many cancer-related **mutations** have been detected. Once the role of oncogenes and proto-oncogenes in cancer was understood, the task of elucidating the exact mutations, specific breakpoints for translocations, and how protein products are altered in the disease process was undertaken.

Karyotype analysis has been used for many years to identify chromosome abnormalities that are specifically associated with particular types of leukemia and lymphoma aiding in diagnosis and the understanding of prognosis. Now that many of the genes involved in the chromosome rearrangements have been cloned, newer, more effective detection techniques, have been discovered. **FISH**, **fluorescence *in situ* hybridization**, uses molecular probes to detect chromosome rearrangements. Probes are developed to detect deletions or to flank the breakpoints of a translocation. or example, using a dual color system for chronic myelogenous leukemia (CML), a green probe hybridizes just distal to the c-*abl* locus on chromosome 9 and a red probe hybridizes just proximal to the locus on chromosome 22. In the absence of a rearrangement, independent colored signals (two green and two red) are observed. When the rearrangement occurs, two of the fluorescent probes are moved adjacent to one another on one chromosome and their signals merge producing a new color (yellow) that can be easily detected (net result: one green, one red, and one yellow signal).

Other molecular techniques such as Southern blotting and **PCR** are also used for cancer detection and can identify point mutations as well as translocations. These systems are set up such that one series of **DNA** fragments indicate no mutation, and a different size fragment or series of fragments will

be seen if a mutation is present. All of the newer techniques are more sensitive than cytogenetic analysis and can pick up abnormal cell lines occurring at very low frequencies. Clinically, it may be useful to detect the disease in an early stage when there are fewer cancer cells present so that treatment may begin before severe symptoms are experienced. In addition, these techniques aid in detection of minimal residual disease (the presence of low levels of disease after treatment) and may give warning that the disease is returning.

A major breakthrough has come in treatment of diseases caused by oncogenes. The current standard of care for cancer patients has been **chemotherapy** and radiation therapy. This is successful in limiting or eradicating the disease, but, because the whole body is affected by these treatments, there are usually multiple side effects such as hair loss, nausea, fatigue, etc. New drugs are designed to counteract the particular mutation associated with the patient's disease and thus are target specific. This is only possible if the mutation causing the disease is known and a treatment can be developed that inactivates the negative affect of that mutation. Because only one cellular component is affected, negative physical side effects may be reduced.

The most successful of these drugs to date is STI-571, or Gleevec, and was developed for use in patients with chronic myelogenous leukemia (CML). In CML, the proto-oncogene translocation results in overproduction of the enzyme tyrosine kinase. Gleevec is an inhibitor of tyrosine kinase and works at the cellular level to block excess enzyme activity. Although there are several different types of tyrosine kinase in humans, STI-571 is specific to the form produced by the CML mutation and does not affect other members of this enzyme family. The drug is therefore so specific, other cells and tissues in the body are not impacted, and there are few negative side effects resulting in a therapy that is much more tolerable to the patient. Early clinical trials showed such a high degree of success that the trails were terminated early and the drug was FDA approved and released for general use. There is now new evidence to suggest that this drug also may be effective for other diseases, including some types of solid tumors. This is clearly the way drug treatments will be designed in the future. By targeting only the defect and correcting that, a disease can be managed without impairing other aspects of a patient's health or quality of life.

Other types of ongoing research include further elucidation of normal proto-oncogene function and how the oncogenic mutations change cellular regulation. In particular, issues involving **oncogene** impact on apoptosis, programmed cell death, have become an important avenue of investigation. It has been shown that normal cells have a fixed life span but that cancer cells lose this characteristic and exhibit uncontrolled cell growth with aspects of immortality. A better understanding of the role oncogenes play in this process may give insight into additional ways to treat cancer.

See also Fluorescence *in situ* hybridization (FISH); Immunogenetics; Immunologic therapies; Mutations and mutagenesis

OPERON

An operon is a single unit of physically adjacent genes that function together under the control of a single operator **gene**. With respect to **transcription** and **translation**, the genes within an operon code for **enzymes** or proteins that are functionally related and are usually members of a single enzyme system. The operon is under the control of a single gene that is responsible for switching the entire operon "on" or "off." A repressor molecule that is capable of binding to the operator gene and switching it, and consequently the whole operon, off, controls the operator gene. A gene that is not part of the operon produces the repressor molecule. The repressor molecule is itself produced by a regulator gene. The repressor molecule is inactivated by a metabolite or signal substance (effector). In other words, the effector causes the operon to become active.

The *lac* operon in the bacterium *E. coli* was one of the first discovered and still remains one of the most studied and well known. The **deoxyribonucleic acid** (**DNA**) segment containing the *lac* operon is some 6,000 base pairs long. This length includes the operator gene and three structural genes (*lac* Z, *lac* Y, and *lac* A). The three structural genes and the operator are transcribed into a single piece of messenger **ribonucleic acid** (mRNA), which can then be translated. Transcription will not take place if a repressor protein is bound to the operator. The repressor protein is encoded by *lac* I, which is a gene located to the left of the *lac* promoter. The *lac* promoter is located immediately to the left of the *lac* operator gene and is outside the *lac* operon. The enzymes produced by this operon are responsible for the hydrolysis (a reaction that adds a water molecule to a reactant and splits the reactant into two molecules) of lactose into glucose and galactose. Once glucose and galactose have been produced, a side reaction occurs forming a compound called allolactose. Allolactose is the chemical responsible for switching on the *lac* operon by binding to the repressor and inactivating it.

Operons are generally encountered in lower organisms such as **bacteria**. They are commonly encountered for certain systems, suggesting that there is a strong evolutionary pressure for the genes to remain together as a unit. Operons have not yet been found in higher organisms, such as multicellular life forms.

A mutation in the operator gene that renders it non-functional would also render the whole operon inactive. As a direct result of inactivation, the coded pathway would no longer operate within the cell. Even though the genes are still separate individual units, they cannot function by themselves, without the control of the operator gene.

See also Genetic code; Microbial genetics

OPSONIZATION

Opsonization is a term that refers to an immune process where particles such as **bacteria** are targeted for destruction by an immune cell known as a **phagocyte**. The process of opsoniza-

tion is a means of identifying the invading particle to the phagocyte. Without the opsonization process the recognition and destruction of invading agents such as bacteria would be inefficient.

The process of opsonization begins when the **immune system** recognizes a particle (e.g., a bacterium) as an invader. The recognition stimulates the production of antibodies that are specific for the antigenic target. Certain **antibody** molecules are stimulated to bind to the surface of the particle. Typically, the binding molecules are a type of antibody classified as IgG. As well, proteins involved in the complement-mediated clearance of foreign material, specifically a protein designated C3b, can bind to the surface of the foreign object. Proteins such as IgG and C3b, which can promote opsonization, are designated as opsonins.

When the IgG antibodies bind to the invading bacterium, the binding is in a specific orientation. An antibody is somewhat "Y" shaped. The binding of IgG to the bacterium is via the branching arms of the "Y." The stalk of the molecule, which is termed the Fc region, then protrudes from the surface. The Fc region is recognized by a receptor on the surface of an immune cell called a phagocyte. When the Fc region is bound to the phagocytic receptor the invading particle is taken into the phagocyte and enzymatically digested.

The Cb3 **complement** protein can bind in a nonspecific manner to an invading particle. Phagocytes also contain surface receptors that recognize and bind Cb3. As with IgG, the binding of Cb3 to the phagocytes triggers a process whereby the invading particle is engulfed, surrounded, and taken inside the phagocytic cell for destruction.

Examples of phagocytic cells that can participate in opsonization are neutrophils and monocytes.

Bacteria that are associated with the development of infections typically possess a capsule, which is a layer of carbohydrate material. The capsular material encases the bacterial cell. The carbohydrate is not recognized as readily by the immune machinery of the body as is protein. As well, the penetration of antibodies through the capsule network to the surface of the bacterium is impeded. Thus, possession of a capsule can dampen the opsonization response.

See also Complement; Immunoglobulins and immunoglobulin deficiency syndromes; Immunity, active, passive and delayed

OPTIC INFECTIONS, CHRONIC

• *see* EYE INFECTIONS

OPTICAL DENSITY AND MEASUREMENTS OF

• *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

ORIGIN OF LIFE

• *see* LIFE, ORIGIN OF

OTIC INFECTIONS, CHRONIC

• *see* EAR INFECTIONS, CHRONIC

OXIDATION-REDUCTION REACTION

Oxidation-reduction reactions are significant to physiological reactions and biochemical pathways important to **microorganisms** and immune processes.

The term oxidation was originally used to describe reactions in which an element combines with oxygen. In contrast, reduction meant the removal of oxygen. By the turn of this century, it became apparent that oxidation always seemed to involve the loss of electrons and did not always involve oxygen. In general, oxidation- reduction reactions involve the exchange of electrons between two species.

An oxidation reaction is defined as the loss of electrons, while a reduction reaction is defined as the gain of electrons. The two reactions always occur together and in chemically equivalent quantities. Thus, the number of electrons lost by one species is always equal to the number of electrons gained by another species. The combination of the two reactions is known as a redox reaction. Species that participate in redox reactions are described as either reducing or oxidizing agents. An oxidizing agent is a species that causes the oxidation of another species. The oxidizing agent accomplishes this by accepting electrons in a reaction. A reducing agent causes the reduction of another species by donating electrons to the reaction.

In general, a strong oxidizing agent is a species that has an attraction for electrons and can oxidize another species. The standard voltage reduction of an oxidizing agent is a measure of the strength of the oxidizing agent. The more positive the species' standard reduction potential, the stronger the species is as an oxidizing agent.

In reactions where the reactants and products are not ionic, there is still a transfer of electrons between species. Chemists have devised a way to keep track of electrons during chemical reactions where the charge on the atoms is not readily apparent. Charges on atoms within compounds are assigned oxidation states (or oxidation numbers). An oxidation number is defined by a set of rules that describes how to divide up electrons shared within compounds. Oxidation is defined as an increase in oxidation state, while reduction is defined as a decrease in oxidation state. Because an oxidizing agent accepts electrons from another species, a component atom of the oxidizing agent will decrease in oxidation number during the redox reaction.

There are many examples of oxidation-reduction reactions in the world. Important processes that involve oxidation-reduction reactions include combustion reactions that convert energy stored in fuels into thermal energy, the corrosion of metals, and metabolic reactions.

Oxidation-reduction reaction occur in both physical and biological settings (where carbon-containing compounds such as carbohydrates are oxidized). The burning of natural gas is an oxidation-reduction reaction that releases energy [$CH_4(g) + 2O_2(g) \rightarrow CO_2(g) + 2H_2O(g) + \text{energy}$]. Redox reactions burn carbohydrates that provide energy [$C_6H_{12}O_6(aq) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l)$]. In both examples, the carbon-containing compound is oxidized, and the oxygen is reduced.

See also Biochemistry

Life on Earth, made possible because of oxygen.

OXYGEN CYCLE IN MICROORGANISMS

The oxygen cycle is a global cycle of oxygen circulation between living organisms and the non-living environment. **Microorganisms** are an important facet of this cycle.

There is substantial evidence in the fossil record that the present atmosphere is due to the activity of **bacteria**, in particular to the bacteria known as cyanobacteria. Originally, the Earth's atmosphere was virtually oxygen-free. With the **evolution** of cyanobacteria, which derive their energy from **photosynthesis** with the subsequent release of oxygen, the oxygen level in the atmosphere increased. Over millions of years of bacterial (and later plant) activity, the oxygen content attained the present day level. Microorganisms such as the cyanobacteria are thus considered producers of atmospheric oxygen.

Microorganisms are also involved in the removal of oxygen from the atmosphere (i.e., they are consumers of oxygen) The process of **respiration** uses oxygen to produce energy. For example, the decay of organic material by microorganisms such as bacteria and **fungi** consumes oxygen. The microbial decomposition process involves numerous species of bacteria and fungi. Some of these release oxygen.

Microorganisms also contribute to the oxygen cycle in an indirect way. For example, the degradation of organic compounds (e.g., cellulose) by bacteria can make the compounds capable of being used as a food source by another organism. This subsequent utilization can both consume and produce oxygen at various stages of the digestive process.

The oxygen cycle in microorganisms also operates at a much smaller scale. The best example of this is the stratification of microbial life in water that occurs due to the oxygen concentration. Oxygen does not dissolve easily in water. Thus, oxygen from the atmosphere enters water very slowly. In a body of water—for example, a lake—the result is a higher concentration of oxygen in the uppermost region of the water. Those bacteria that produce oxygen (i.e., cyanobacteria) will also be located in this surface region of the water, because Sunlight is most available there. Food sources that are not consumed by these bacteria and other surface-dwelling life sink to deeper water. In the deeper water, bacteria and other microorganisms that can live in the presence of low oxygen levels then utilize the nutrients. At the greatest depths reside microorganisms that cannot tolerate oxygen. These anaerobic microorganisms degrade the nutrients that reach the bottom. This stratification of microbial life will affect the presence of other life in the water, as well as the cycling of other compounds (e.g., the carbon cycle).

The oxygen cycle in microorganisms in bodies of water such as lakes and rivers can have important consequences on the health of the water. For example, if mixing of the water in

the lake does not readily occur, the body of water can become stagnant. In other words, the oxygen content of the water becomes depleted and, without mixing, insufficient surface level oxygen is available to replenish the supply. Fish life in the water can suffer. Another example is the depletion of oxygen from a lake by the explosive growth of algae. The algal "bloom" can essentially make the water body incapable of supporting life. Furthermore, if the algal species is a toxin producer, the water can become hazardous to health. A final example is the relationship between the oxygen cycle in microorganisms and **water pollution**. Polluted water is typically enriched in nutrients that will support the rapid growth of bacteria and other microbes. Their growth depletes the oxygen in the water. In grossly polluted water, this depletion can be so extensive that the water cannot support oxygen-dependent life.

Thus, the oxygen cycle in microorganisms, mainly bacteria, is very important in determining the quality of a water body and so of the ability of the water to be a productive source of life.

See also Carbon cycle in microorganisms; Composting, microbiological aspects; Life, origin of; Nitrogen cycle in microorganisms

P

PAEOPHYTA

Also known as brown algae, Paeophyta (or Phaeophyta) are photosynthetic **protists**, belonging to the Chromista Kingdom (i.e., "with color"), a kingdom closely related neither to plants nor to other algae. This kingdom includes microscopic life forms such as **diatoms**, colorless mildews, giant kelps, and sargassum. Most Chromista are photosynthetic, including Paeophyta, but they also make other pigments not found in plants, including a modified **chlorophyll** with a different molecular shape from that synthesized by plants. Paeophytes also make high levels of carotenoids, in special fucoxanthin, which give them their golden and brown colors. Unlike plants, they do not store energy as glucose and starch, but as laminarin, a polymer formed by glucose and a six-carbon sugar alkaloid termed mannitol. Most paeophytes reproduce through sexual alternation of generations, with some species presenting a dominant diploid phase (such as kelps) and others isomorphic phases (i.e., each generation being very similar to each other).

Paeophyta comprises several genera, including the largest species among the Chromista, although many species are microscopic brown algae, which grow on underwater rock or coral surfaces, or on vegetation, forming encrustations or filamentous networks, such as those commonly found in and around underwater giant kelp forests. Giant kelps form dense sea forests such as those found in the tidepools nearby Monterrey, California, with long and strong stalks up to 50–60 meters (197 feet) high, fixed at the sea bottom through brushy holdfasts. From the stalks grow flat blades termed lamina that capture sunlight and make **photosynthesis**. Some **kelp** have flotation bladders that sustain their photosynthetic blades near the water surface, for better exposition to solar energy. Paeophytes grow in coastal marine cold and temperate water, with a few species growing in freshwaters as well. Many are intertidal species, and are exposed to open air during low tide, such as Fucus (rockweed). Some Paeophytes, such as *Sargassum natans* and *Sargassum fluitans* are pelagic species (i.e., free-floating species), due to their gas-filled vesicles. They form floating ecosystems in the western North Atlantic sea that support more than 50 different species of fish and several species of crabs, as well as invertebrates, such as gastropods, polychaetes, anemones, sea-spiders, etc.

See also Photosynthetic microorganisms

PANDEMICS • *see* EPIDEMICS AND PANDEMICS

PARAMECIUM

Paramecium are single celled **eukaryotes**, reminiscent of a football in shape, that belong to the group of **microorganisms** known as the **Protozoa**. The protozoan inhabits freshwater bodies such as ponds. The organism is useful as a teaching tool for light microscopy.

There are at least eight species of *Paramecium*. Two examples are *Paramecium caudatum* and *Paramecium bursaria*.

Paramecium are large enough to be visible to the unaided eye. However, the internal detail is resolved only by the use of a **microscope**. A student is best able to observe the complex internal organization of the organism by using what is termed the hanging drop technique. Here a drop of water is suspended upside-down on a cover slip that is positioned over a cavity on a microscope slide. The cover slip is sealed to prevent leakage.

Paramecium contain organized structures called vacuoles that are essentially a primitive mouth, stomach, and excretion system. As food enters the organism, it is stored in specialized vacuoles known as food vacuoles. These can circulate through the **cytoplasm** of the organism, in order to provide food where needed. Characteristic of eukaryotes, nuclear material is segregated by a nuclear membrane.

Another characteristic features of *Paramecium* is the so-called contractile vacuole. This vacuole is able to store water and then, by virtue of the compression of the side arms that radiate from the central vacuole, to expel the water out of the

Light micrograph of a paramecium.

organism. In this way, the amount of water inside the paramecia can be controlled. The operation of the contractile vacuole is another feature that is visible by the light microscopic observation of living paramecia.

On the exterior lies a membrane that is called the pellicle. The pellicle is both stiff, to provide support and to maintain the shape of the organism, and is flexible, to allow some flexing of the surface. Also on the surface are hundreds of tiny hairs called cilia. The cilia wave back and forth, and act to sweep food particles (**bacteria** and smaller protozoa) towards the primitive mouth of the organism (the gullet). The cilia are also important in locomotion, acting analogous to the oars of a rowboat. The beating of the cilia is easily visible under light microscopic examination, especially if the movement of the organism has been retarded by the addition of a viscous compound such as glycerol to the sample.

See also Eukaryotes

PARASITES

A parasite is an organism that depends upon another organism, known as a host, for food and shelter. The parasite usually gains all the benefits of this relationship, while the host may suffer from various diseases and discomforts, or show no signs of the infection. The life cycle of a typical parasite usually includes several developmental stages and morphological changes as the parasite lives and moves through the environment and one or more hosts. Parasites that remain on a host's body surface to feed are called ectoparasites, while those that live inside a host's body are called endoparasites. Parasitism is a highly successful biological adaptation. There are more known parasitic species than nonparasitic ones, and parasites affect just about every form of life, including most all animals, plants, and even **bacteria**.

Parasitology is the study of parasites and their relationships with host organisms. Throughout history, people have coped with over 100 types of parasites affecting humans. Parasites have not, however, been systematically studied until the last few centuries. With his invention of the **microscope** in the late 1600s, Anton von Leeuwenhoek was perhaps the first to observe microscopic parasites. As Westerners began to travel and work more often in tropical parts of the world, medical researchers had to study and treat a variety of new infections, many of which were caused by parasites. By the early 1900s, parasitology had developed as a specialized field of study.

Typically, a parasitic infection does not directly kill a host, though the drain on the organism's resources can affect its growth, reproductive capability, and survival, leading to premature death. Parasites, and the diseases they cause and transmit, have been responsible for tremendous human suffering and loss of life throughout history. Although the majority of parasitic infections occur within tropical regions and among low-income populations, most all regions of the world sustain parasitic species, and all humans are susceptible to infection.

Although many species of **viruses**, bacteria, and **fungi** exhibit parasitic behavior and can be transmitted by parasites, scientists usually study them separately as infectious diseases. Types of organisms that are studied by parasitologists include species of **protozoa**, helminths or worms, and arthropods.

Protozoa are one-celled organisms that are capable of carrying out most of the same physiological functions as multicellular organisms by using highly developed organelles within their cell. Many of the over 45,000 species of known protozoa are parasitic. As parasites of humans, this group of organisms has historically been the cause of more suffering and death than any other category of disease causing organisms.

Intestinal protozoa are common throughout the world and particularly in areas where food and water sources are subject to **contamination** from animal and human waste. Typically, protozoa that infect their host through water or food do so while in an inactive state, called a cyst, where they have encased themselves in a protective outer membrane, and are released through the digestive tract of a previous host. Once inside the host, they develop into a mature form that feeds and reproduces.

Amebic dysentery is one of the more common diseases that often afflicts travelers who visit tropical and sub-tropical regions. This condition, characterized by diarrhea, vomiting and weakness, is caused by a protozoan known as *Entamoeba histolytica*. Another protozoan that causes severe diarrhea, but is also found in more temperate regions, is *Giardia lamblia*. Among Leeuwenhoek's discoveries was *G. lamblia,* which is a now well-publicized parasite that can infect hikers who drink untreated water in the back country.

Other types of parasitic protozoa infect the blood or tissues of their hosts. These protozoa are typically transmitted through another organism, called a vector, which carries the parasite before it enters the final host. Often the vector is an invertebrate, such as an insect, that itself feeds on the host and passes the protozoan on through the bite wound. Some of the most infamous of these protozoa are members of the genera *Plasmodium,* that cause **malaria**; *Trypanosoma,* that cause African **sleeping sickness**; and *Leishmania,* which leads to a number of debilitating and disfiguring diseases.

Helminths are worm-like organisms of which several classes of parasites are found including nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). Leeches, of the phylum Annelid, are also helminths and considered as ectoparasitic, attaching themselves to the outside skin of their hosts. Nematodes, or roundworms, have an estimated 80,000 species that are known to be parasitic. The general morphology of these worms is consistent with their name; they are usually long and cylindrical in shape. One of the most infamous nematodes is *Trichinella spiralis,* a parasite that lives its larval stage encysted in the muscle tissue of animals, including swine, and make their way into the intestinal tissue of humans who happen to digest infected, undercooked pork.

Arthropods are organisms characterized by exoskeletons and segmented bodies such as crustaceans, insects, and arachnids. They are the most diverse and widely distributed animals on the planet. Many arthropod species serve as carriers of bacterial and viral diseases, as intermediate hosts for protozoan and helminth parasites, and as parasites themselves.

Certain insect species are the carriers of some of humanity's most dreaded diseases, including malaria, **typhus**, and plague. As consumers of agricultural crops and parasites of our livestock, insects are also humankind's number one competitor for resources.

Mosquitoes, are the most notorious carriers, or vectors, of disease and parasites. Female mosquitoes rely on warm-blooded hosts to serve as a blood meal to nourish their eggs. During the process of penetrating a host's skin with their long, sucking mouth parts, saliva from the mosquito is transferred into the bite area. Any viral, protozoan, or helminth infections carried in the biting mosquito can be transferred directly into the blood stream of its host. Among these are malaria, **yellow fever**, *W. bancrofti* (filariasis and elephantiasis), and *D. immitis* (heartworm).

Flies also harbor diseases that can be transmitted to humans and other mammals when they bite to obtain a blood meal for themselves. For example, black flies can carry river blindness, sandflies can carry leishmaniasis and kala-azar, and tsetse flies, found mainly in Africa, carry the trypanosomes that cause sleeping sickness. Livestock, such as horses and cattle, can be infected with a variety of botflies and warbles that can infest and feed on the skin, throat, nasal passages, and stomachs of their hosts.

Fleas and lice are two of the most common and irritating parasitic insects of humans and livestock. Lice commonly live among the hairs of their hosts, feeding on blood. Some species are carriers of the epidemic inducing typhus fever. Fleas usually infest birds and mammals, and can feed on humans when they are transferred from pets or livestock. Fleas are known to carry a variety of devastating diseases, including the plague.

Another prominent class of arthropods that contains parasitic species is the arachnids. Though this group is more commonly known for spiders and scorpions, its parasitic members include ticks and mites. Mites are very small arachnids that infest both plants and animals. One common type is chiggers, which live in grasses and, as larva, grab onto passing animals and attach themselves to the skin, often leading to irritating rashes or bite wounds. Ticks also live their adult lives among grasses and short shrubs. They are typically larger than mites, and it is the adult female that attaches itself to an animal host for a blood meal. Tick bites themselves can be painful and irritating. More importantly, ticks can carry a number of diseases that affect humans. The most common of these include Rocky Mountain spotted fever, Colorado tick fever, and the latest occurrence of tick-borne infections, **Lyme disease**.

Most parasitic infections can be treated by use of medical and surgical procedures. The best manner of controlling infection, though, is prevention. Scientists have developed and continue to test a number of drugs that can be taken as a barrier, or prophylaxis, to certain parasites. Other measures of control include improving sanitary conditions of water and food sources, proper cooking techniques, education about personal **hygiene**, and control of intermediate and vector host organisms.

PARKMAN, PAUL DOUGLAS (1932-)

American physician

Paul Parkman isolated the rubella (German **measles**) virus and, with Harry Martin Meyer (1928–2001), co-discovered the first widely applicable test for rubella antibodies and the **vaccine** against rubella.

Born in Auburn, New York, on May 29, 1932, the son of Stuart Douglas Parkman, a postal clerk, and his wife Mary née Klumpp, a homemaker, Parkman graduated from Weedsport, New York, High School in 1950. His father also served on the Weedsport Central School Board of Education and raised turkeys and chickens to help finance his son's education. Parkman took advantage of a special three-year premedical program at St. Lawrence University, majored in biology, and received both his M.D. from the State University of New York Upstate Medical Center College of Medicine (now Upstate Medical University) and his B.S. from St. Lawrence together in 1957.

After his internship at Mary Imogene Bassett Hospital, Cooperstown, New York, from 1957 to 1958, and his residency in pediatrics at the Upstate Medical Center Hospitals from 1958 to 1960, Parkman joined the army and was assigned to Walter Reed Army Medical Center, Washington, D.C. In 1963, he began working for the Division of Biologics Standards, National Institutes of Health (NIH), as a virologist. From 1963 to 1972, he was chief of the Section of General **Virology** in the Laboratory for Viral **Immunology** at the Division of Biologics Standards. In 1973, the Division of Biologics Standards was transferred to the Food and Drug Administration (FDA), where Parkman remained until he retired from federal government service on July 31, 1990. He served the FDA as director of the Division of Virology and from 1973 to 1987 in a variety of roles within the Bureau of Biology and the Center for Drugs and Biologics. From 1987 to 1990, he was the founding director of the Center for Biologics Evaluation. After his retirement, he remained in Kensington, Maryland, to consult on biological products, especially vaccines.

At Walter Reed in 1960, Parkman and his associates Edward Louis Buescher (b. 1925) and Malcolm Stewart Artenstein (b. 1930) found and used an opportunity to study rubella, which they noticed was common among military recruits. Simultaneously working on the same problem were Thomas Huckle Weller (b. 1915) and Franklin Allen Neva (b. 1922) at Harvard Medical School. In 1962 the two teams independently succeeded in isolating the virus, a member of the Togaviridae family, and each published its results in the same volume of the *Proceedings of the Society of Experimental Biology and Medicine*.

Upon developing the first reliable test for rubella antibodies, thus making accurate diagnosis of the disease possible, Parkman immediately began to create a vaccine from the attenuated virus. Meyer, Parkman, and Theodore Constantine Panos (b. 1915) reported successful clinical trials of their vaccine in the *New England Journal of Medicine* in 1966. The last major rubella epidemic was in 1964. In the 1970s, the rubella vaccine became a component of the measles-mumps-rubella vaccine (MMR), now commonly administered to children at 15 months.

See also Immunization; Virology

PASSIVE DIFFUSION • *see* CELL MEMBRANE TRANSPORT

PASTEUR, LOUIS (1822-1895)

French chemist

Louis Pasteur left a legacy of scientific contributions that include an understanding of how **microorganisms** carry on the biochemical process of **fermentation**, the establishment of the causal relationship between microorganisms and disease, and the concept of destroying microorganisms to halt the transmission of communicable disease. These achievements led him to be called the founder of microbiology.

After his early education, Pasteur went to Paris to study at the Sorbonne, then began teaching chemistry while still a student. After being appointed chemistry professor at a new university in Lille, France, Pasteur began work on **yeast** cells and showed how they produce alcohol and carbon dioxide from sugar during the process of fermentation. Fermentation is a form of cellular **respiration** carried on by yeast cells, a way of getting energy for cells when there is no oxygen present. Pasteur found that fermentation would take place only when living yeast cells were present.

Establishing himself as a serious, hard-working chemist, Pasteur was called upon to tackle some of the problems plaguing the French beverage industry at the time. Of special concern was the spoiling of wine and beer, which caused great economic loss, and tarnished France's reputation for fine vintage wines. Vintners wanted to know the cause of l'amer, a condition that was destroying France's best burgundy wines. Pasteur looked at wine under the **microscope** and noticed that when aged properly, the liquid contained little spherical yeast cells. But when the wine turned sour, there was a proliferation of bacterial cells that produced lactic acid. Pasteur suggested that heating the wine gently at about 120°F (49°C) would kill the **bacteria** that produced lactic acid and let the wine age properly. Pasteur's book *Etudes sur le Vin*, published in 1866, was a testament to two of his great passions—the scientific method and his love of wine. It caused another

French revolution—one in winemaking, as Pasteur suggested that greater cleanliness was need to eliminate bacteria and that this could be accomplished using heat. Some wine-makers were initially reticent to heat their wines, but the practice eventually saved the wine industry in France.

The idea of heating to kill microorganisms was applied to other perishable fluids, including milk, and the idea of **pasteurization** was born. Several decades later in the United States, the pasteurization of milk was championed by American bacteriologist Alice Catherine Evans, who linked bacteria in milk with the disease **brucellosis**, a type of fever found in different variations in many countries.

In his work with yeast, Pasteur also found that air should be kept from fermenting wine, but was necessary for the production of vinegar. In the presence of oxygen, yeasts and bacteria break down alcohol into acetic acid, or vinegar. Pasteur also informed the vinegar industry that adding more microorganisms to the fermenting mixture could increase vinegar production. Pasteur carried on many experiments with yeast. He showed that fermentation can take place without oxygen (anaerobic conditions), but that the process still involved living things such as yeast. He did several experiments to show (as Lazzaro Spallanzani had a century earlier) that living things do not arise spontaneously but rather come from other living things. To disprove the idea of spontaneous generation, Pasteur boiled meat extract and left it exposed to air in a flask with a long S-shaped neck. There was no decay observed because microorganisms from the air did not reach the extract. On the way to performing his experiment Pasteur had also invented what has come to be known as sterile technique, boiling or heating of instruments and food to prevent the proliferation of microorganisms.

In 1862, Pasteur was called upon to help solve a crisis in another ailing French industry. The silkworms that produced silk fabric were dying of an unknown disease. Armed with his microscope, Pasteur traveled to the south of France in 1865. Here Pasteur found the tiny **parasites** that were killing the silkworms and affecting their food, mulberry leaves. His solution seemed drastic at the time. He suggested destroying all the unhealthy worms and starting with new cultures. The solution worked, and soon French silk scarves were back in the marketplace.

Pasteur then turned his attention to human and animal diseases. He supposed for some time that microscopic organisms cause disease and that these tiny microorganisms could travel from person to person spreading the disease. Other scientists had expressed this thought before, but Pasteur had more experience using the microscope and identifying different kinds of microorganisms such as bacteria and **fungi**.

In 1868, Pasteur suffered a stroke and much of his work thereafter was carried out by his wife Marie Laurent Pasteur. After seeing what military hospitals were like during the Franco-Prussian War, Pasteur impressed upon physicians that they should boil and sterilize their instruments. This was still not common practice in the nineteenth century.

Pasteur developed techniques for culturing and examining several disease-causing bacteria. He identified *Staphylococcus pyogenes* bacteria in boils and *Streptococcus pyogenes* in puerperal fever. He also cultured the bacteria that cause cholera. Once when injecting healthy chickens with cholera bacteria, he expected the chickens to become sick. Unknown to Pasteur, the bacteria were old and no longer virulent. The chickens failed to get the disease, but instead they received **immunity** against cholera. Thus, Pasteur discovered that weakened microbes make a good **vaccine** by imparting immunity without actually producing the disease.

Louis Pasteur, who refuted the theory of spontaneous generation and developed the sterilization technique of pasteurization.

Pasteur then began work on a vaccine for **anthrax**, a disease that killed many animals and infected people who contracted it from their sheep and thus was known as "wool sorters' disease." Anthrax causes sudden chills, high fever, pain, and can affect the brain. Pasteur experimented with weakening or attenuating the bacteria that cause anthrax, and in 1881 produced a vaccine that successfully prevented the deadly disease.

Pasteur's last great scientific achievement was developing a successful treatment for **rabies**, a deadly disease contracted from bites of an infected, rabid animal. Rabies, or hydrophobia, first causes pain in the throat that prevents swallowing, then brings on spasms, fever, and finally death. Pasteur knew that rabies took weeks or even months to

become active. He hypothesized that if people were given an injection of a vaccine after being bitten, it could prevent the disease from manifesting. After methodically producing a rabies vaccine from the spinal fluid of infected rabbits, Pasteur sought to test it. In 1885, nine-year-old Joseph Meister, who had been bitten by a rabid dog, was brought to Pasteur, and after a series of shots of the new rabies vaccine, the boy did not develop any of the deadly symptoms of rabies.

To treat cases of rabies, the Pasteur Institute was established in 1888 with monetary donations from all over the world. It later became one of the most prestigious biological research institutions in the world. When Pasteur died in 1895, he was well recognized for his outstanding achievements in science.

See also Bacteria and bacterial infection; Colony and colony formation; Contamination, bacterial and viral; Epidemiology, tracking diseases with technology; Epidemiology; Food preservation; Germ theory of disease; History of microbiology; History of public health; Immunogenetics; Infection control; Winemaking

PASTEURELLA

Pasteurella is a genus, or subdivision, of **bacteria**. The genus is in turn a member of the family Pasteurellaceae, which includes the genus Hemophilus. Members of this genus *Pasteurella* are short rod-shaped bacteria that produce the negative reaction in the Gram stain procedure, are incapable of the active type of movement called motility, and can grow both in the presence and the absence of oxygen.

Pasteurella causes diseases in humans and many species of animals. One species in particular, *Pasteurella multocida* causes disease in both humans and animals. For example, almost all pet rabbits will at one time or another acquire infections of the nose, eyes, and lungs, or develop skin sores because of a *Pasteurella multocida* infection. The bacterium also causes a severe infection in poultry, including lameness and foul cholera, and illness in cattle and swine. Another species, *Pasteurella pneumotrophica*, infects mice, rats, guinea pigs, hamsters, and other animals that are often used in laboratory studies.

The annual economic cost of the losses due to these infections are several hundred million dollars in the United States alone.

In humans, *Pasteurella multocida* can be acquired from the bite of a cat or dog. From 20% to 50% of the one to two million Americans, mostly children, who are bitten by dogs and cats each year will develop the infection. Following some swelling at the site of the bite, the bacteria can migrate. An infection becomes established in nearby joints, where it produces swelling, arthritis, and pain.

Infections respond to common **antibiotics** including **penicillin**, tetracycline, and chloramphenicol. Despite the relative ease of treatment of the infection, little is still known of the genetic basis for the ability of the bacteria to establish an infection, and of the factors that allow the bacterium to evade the defense mechanisms of the host. In the controlled conditions of the laboratory, the adherent populations known as biofilms can be formed by *Pasteurella multocida*.

The recent completion of the genetic sequence of *Pasteurella multocida* will aid in determining the genes, and so their protein products, which are critical for infection.

See also Bacteria and bacterial infection; Proteomics

PASTEURIZATION

Pasteurization is a process whereby fluids such as wine and milk are heated for a predetermined time at a temperature that is below the boiling point of the liquid. The treatment kills any **microorganisms** that are in the fluid but does not alter the taste, appearance, or nutritive value of the fluid.

The process of pasteurization is named after the French chemist **Louis Pasteur** (1822–1895), who is regarded as the founder of the study of modern microbiology. Among Pasteur's many accomplishments was the observation that the heating of fluids destroys harmful **bacteria**.

The basis of pasteurization is the application of heat. Many bacteria cannot survive exposure to the range of temperatures used in pasteurization. The energy of the heating process is disruptive to the membrane(s) that enclose the bacteria. As well, the bacterial **enzymes** that are vital for the maintenance of the growth and survival of the bacteria are denatured, or lose their functional shape, when exposed to heat. The disruption of bacteria is usually so complete that recovery of the cells following the end of the heat treatment is impossible.

The pasteurization process is a combination of temperature, time, and the consistency of the product. Thus, the actual conditions of pasteurization can vary depending on the product being treated. For example heating at 145°F (63°C) for not less than 30 minutes or at 162°F (72°C) for not less than 16 seconds pasteurizes milk. A product with greater consistency, such ice cream or egg nog, is pasteurized by heating at a temperature of at least 156°F (69°C) for not less than 30 minutes or at a temperature of at least 176°F (80°C) for not less than 25 seconds.

Particularly in commercial settings, such as a milk processing plant, there are two long-standing methods of pasteurization. These are known as the batch method and the continuous method. In the batch method the fluid is held in one container throughout the process. This method of pasteurization tends to be used for products such as ice cream. Milk tends to be pasteurized using the continuous method.

In the continuous method the milk passes by a stack of steel plates that are heated to the desired temperature. The flow rate is such that the milk is maintained at the desired temperature for the specified period of time. The pasteurized milk then flows to another tank.

Several other more recent variations on the process of pasteurization have been developed. The first of these variations is known as flash pasteurization. This process uses a higher temperature than conventional pasteurization, but the temperature is maintained for a shorter time. The product is

then rapidly cooled to below 50°F (10°C), a temperature at which it can then be stored. The intent of flash pasteurization is to eliminate harmful microorganisms while maintaining the product as close as possible to its natural state. Juices are candidates for this process. In milk, **lactic acid bacteria** can survive. While these bacteria are not a health threat, their subsequent metabolic activity can cause the milk to sour.

Another variation on pasteurization is known as ultra-pasteurization. This is similar to flash pasteurization, except that a higher than normal pressure is applied. The higher pressure greatly increases the temperature that can be achieved, and so decreases the length of time that a product, typically milk, needs to be exposed to the heat. The advantage of ultra-pasteurization is the extended shelf live of the milk that results. The milk, which is essentially sterile, can be stored unopened at room temperature for several weeks without compromising the quality.

In recent years the term cold pasteurization has been used to describe the **sterilization** of solids, such as food, using radiation. The applicability of using the term pasteurization to describe a process that does not employ heat remains a subject of debate among microbiologists.

Pasteurization is effective only until the product is exposed to the air. Then, microorganisms from the air can be carried into the product and growth of microorganisms will occur. The chance of this **contamination** is lessened by storage of milk and milk products at the appropriate storage temperatures after they have been opened. For example, even ultra-pasteurized milk needs to stored in the refrigerator once it is in use.

See also Bacteriocidal, bacteriostatic; Sterilization

PATHOGEN • *see* MICROBIOLOGY, CLINICAL

PENICILLIN

One of the major advances of twentieth-century medicine was the discovery of penicillin. Penicillin is a member of the class of drugs known as **antibiotics**. These drugs either kill (bacteriocidal) or arrest the growth of (bacteriostatic) **bacteria** and **fungi** (**yeast**), as well as several other classes of infectious organisms. Antibiotics are ineffective against **viruses**. Prior to the advent of penicillin, bacterial infections such as **pneumonia** and sepsis (overwhelming infection of the blood) were usually fatal. Once the use of penicillin became widespread, fatality rates from pneumonia dropped precipitously.

The discovery of penicillin marked the beginning of a new era in the fight against disease. Scientists had known since the mid-nineteenth century that bacteria were responsible for some infectious diseases, but were virtually helpless to stop them. Then, in 1928, **Alexander Fleming** (1881–1955), a Scottish bacteriologist working at St. Mary's Hospital in London, stumbled onto a powerful new weapon.

Fleming's research centered on the bacteria *Staphylococcus*, a class of bacteria that caused infections such as pneumonia, abscesses, post-operative wound infections, and sepsis. In order to study these bacteria, Fleming grew them in his laboratory in glass Petri dishes on a substance called **agar**. In August, 1928 he noticed that some of the Petri dishes in which the bacteria were growing had become contaminated with **mold**, which he later identified as belonging to the Penicillum family.

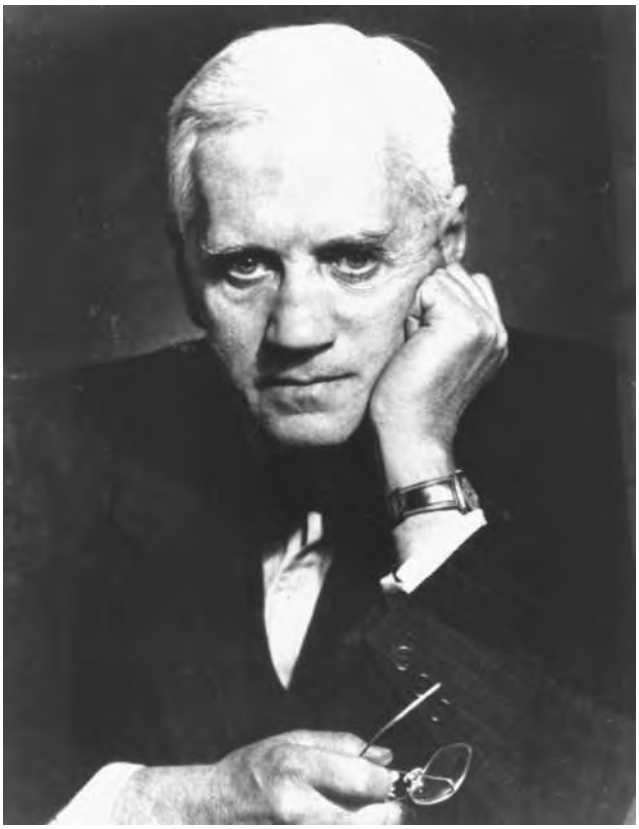
Fleming noted that bacteria in the vicinity of the mold had died. Exploring further, Fleming found that the mold killed several, but not all, types of bacteria. He also found that an extract from the mold did not damage healthy tissue in animals. However, growing the mold and collecting even tiny amounts of the active ingredient—penicillin—was extremely difficult. Fleming did, however, publish his results in the medical literature in 1928.

Ten years later, other researchers picked up where Fleming had left off. Working in Oxford, England, a team led by Howard Florey (1898–1968), an Australian, and Ernst Chain, a refugee from Nazi Germany, came across Fleming's study and confirmed his findings in their laboratory. They also had problems growing the mold and found it very difficult to isolate the active ingredient

Another researcher on their team, Norman Heatley, developed better production techniques, and the team was able to produce enough penicillin to conduct tests in humans. In 1941, the team announced that penicillin could combat disease in humans. Unfortunately, producing penicillin was still a cumbersome process and supplies of the new drug were extremely limited. Working in the United States, Heatley and other scientists improved production and began making large quantities of the drug. Owing to this success, penicillin was available to treat wounded soldiers by the latter part of World War II. Fleming, Florey, and Chain were awarded the Noble Prize in medicine. Heatley received an honorary M.D. from Oxford University in 1990.

Penicillin's mode of action is to block the construction of cell walls in certain bacteria. The bacteria must be reproducing for penicillin to work, thus there is always some lag time between dosage and response.

The mechanism of action of penicillin at the molecular level is still not completely understood. It is known that the initial step is the binding of penicillin to penicillin-binding proteins (PBPs), which are located in the cell wall. Some PBPs are inhibitors of cell autolytic **enzymes** that literally eat the cell wall and are most likely necessary during cell division. Other PBPs are enzymes that are involved in the final step of cell wall synthesis called transpeptidation. These latter enzymes are outside the cell membrane and link cell wall components together by joining glycopeptide polymers together to form **peptidoglycan**. The bacterial cell wall owes its strength to layers composed of peptidoglycan (also known as murein or mucopeptide). Peptidoglycan is a complex polymer composed of alternating N-acetylglucosamine and N-acetylmuramic acid as a backbone off of which a set of identical tetrapeptide side chains branch from the N-acetylmuramic acids, and a set of identical peptide cross-bridges also branch. The tetrapeptide side chains and the cross-bridges vary from species to species, but the backbone is the same in all bacterial species.



Sir Alexander Flemming, the discoverer of peniciliin.

Each peptidoglycan layer of the cell wall is actually a giant polymer molecule because all peptidoglycan chains are cross-linked. In gram-positive bacteria there may be as many as 40 sheets of peptidoglycan, making up to 50% of the cell wall material. In Gram-negative bacteria, there are only one or two sheets (about 5–10% of the cell wall material). In general, penicillin G, or the penicillin that Fleming discovered, has high activity against Gram-positive bacteria and low activity against Gram-negative bacteria (with some exceptions).

Penicillin acts by inhibiting peptidoglycan synthesis by blocking the final transpeptidation step in the synthesis of peptidoglycan. It also removes the inactivator of the inhibitor of autolytic enzymes, and the autolytic enzymes then lyses the cell wall, and the bacterium ruptures. This latter is the final bacteriocidal event.

Since the 1940s, many other antibiotics have been developed. Some of these are based on the molecular structure of penicillin; others are completely unrelated. At one time, scientists assumed that bacterial infections were conquered by the development of antibiotics. However, in the late twentieth century, bacterial resistance to antibiotics—including penicillin—was recognized as a potential threat to this success. A classic example is the *Staphylococcus* bacteria, the very species Fleming had found killed by penicillin on his Petri dishes. By 1999, a large percentage of *Staphylococcus* bacteria were resistant to penicillin G. Continuing research so far has been able to keep pace with emerging resistant strains of bacteria. Scientists and physicians must be judicious about the use of antibiotics, however, in order to minimize bacterial resistance and ensure that antibiotics such as penicillin remain effective agents for treatment of bacterial infections.

See also Antibiotic resistance, tests for; Bacteria and bacterial infection; Bacterial adaptation; Bacterial growth and division; Bacterial membranes and cell wall; History of the development of antibiotics

PENNINGER, JOSEF MARTIN (1964-)

Austrian molecular immunologist

Josef Penninger is a medical doctor and molecular immunologist. In his short research career he has already made discoveries of fundamental significance to the understanding of bacterial infections and heart disease, osteoporosis, and the human **immune system**.

Penninger was born in Gurten, Austria. His education was in Austria, culminating with his receipt of a M.D. and Ph.D. from the University of Innsbruck in 1998. In 1990, he joined the Ontario Cancer Institute in Toronto. In 1994, he became principle investigator with the United States **biotechnology** company Amgen, joining the AMEN Research Institute that had just been established at the Department of Medical Biophysics at the University of Toronto.

In his decade at the AMEN Institute, Penninger has produced a steady stream of groundbreaking studies across the breath of **immunology**. He and his colleagues demonstrated that infection with the bacterial *Chlamydia trachomatis* caused heart damage in mice. The basis of the damage is an immune reaction to a bacterial protein that mimics the structure of the protein constituent of the heart valve.

As well, Penninger has shown that a protein called CD45 is responsible for regulating how a body's cells respond to developmental signals, coordinates the functioning of cells such as red and white blood cells, and regulates the response of the immune system to viral infection. The discovery of this key regulator and how it is co-opted in certain diseases is already viewed as a vital step to controlling diseases and preventing the immune system from attacking its own tissues (a response called an autoimmune reaction).

The research of Penninger and others, such as Barry Marshall and Stanley Pruisner, has caused a re-assessment of the nature of certain diseases. Evidence is consistent so far with a bacterial or biological origin for diseases such as schizophrenia, multiple sclerosis and Alzheimer's disease.

Penninger already has some 150 research papers published, many in the world's most prestigious scientific journals. Numerous prizes and distinctions have recognized the scope and importance of his work.

See also Chlamydial pneumonia; Immune system

PEPTIDOGLYCAN

Peptidoglycan is the skeleton of **bacteria**. Present in both Gram-positive and Gram-negative bacteria, the peptidoglycan is the rigid sac that enables the bacterium to maintain its shape.

This rigid layer is a network of two sugars that are cross-linked together by amino acid bridges. The sugars are N-acetyl glucosamine and N-acetyl muramic acid. The latter sugar is unique to the peptidoglycan, and is found no where else in nature.

The peptidoglycan in Gram-negative bacteria is only a single layer thick, and appears somewhat like the criss-cross network of strings on a tennis racket. The layer lies between the two membranes that are part of the cell wall of Gram-negative bacteria, and comprises only about twenty percent of the weight of the cell wall. In Gram-positive bacteria, the peptidoglycan is much thicker, some 40 sugars thick, comprising up to ninety percent of the weight of the cell wall. The cross bridging is three-dimensional in this network. The peptidoglycan layer is external to the single membrane, and together they comprise the cell wall of Gram-positive bacteria.

Research has demonstrated that the growth of the peptidoglycan occurs at sites all over a bacterium, rather than at a single site. Newly made peptidoglycan must be inserted into the existing network in such a way that the strength of the peptidoglycan sheet is maintained. Otherwise, the inner and outer pressures acting on the bacterium would burst the cell. This problem can be thought of as similar to trying to incorporate material into an inflated balloon without bursting the balloon. This delicate process is accomplished by the coordinate action of **enzymes** that snip open the peptidoglycan, insert new material, and bind the old and new regions together. This process is also coordinated with the rate of **bacterial growth**. The faster a bacterium is growing, the more quickly peptidoglycan is made and the faster the peptidoglycan sac is enlarged.

Certain **antibiotics** can inhibit the growth and proper linkage of peptidoglycan. An example is the beta-lactam class of antibiotics (such as **penicillin**). Also, the enzyme called lysozyme, which is found in the saliva and the tears of humans, attacks peptidoglycan by breaking the connection between the sugar molecules. This activity is one of the important bacterial defense mechanisms of the human body.

See also Bacterial ultrastructure

PERIPLASM

The periplasm is a region in the cell wall of Gram-negative **bacteria**. It is located between the outer membrane and the inner, or cytoplasmic, membrane. Once considered to be empty space, the periplasm is now recognized as a specialized region of great importance.

The existence of a region between the membranes of Gram-negative bacteria became evident when **electron microscopic** technology developed to the point where samples could be chemically preserved, mounted in a resin, and sliced very thinly. The so-called thin sections allowed electrons to pass through the sample when positioned in the electron **microscope**. Areas containing more material provided more contrast and so appeared darker in the electron image. The region between the outer and inner membranes presented a white appearance. For a time, this was interpreted as being indicative of a void. From this visual appearance came the notion that the space was functionless. Indeed, the region was first described as the periplasmic space.

Techniques were developed that allowed the outer membrane to be made extremely permeable or to be removed altogether while preserving the integrity of the underlying membrane and another stress-bearing structure called the **peptidoglycan**. This allowed the contents of the periplasmic space to be extracted and examined.

The periplasm, as it is now called, was shown to be a true cell compartment. It is not an empty space, but rather is filled with a periplasmic fluid that has a gel-like consistency. The periplasm contains a number of proteins that perform various functions. Some proteins bind molecules such as sugars, amino acids, vitamins, and ions. Via association with other cytoplasmic membrane-bound proteins these proteins can release the bound compounds, which then can be transported into the **cytoplasm** of the bacterium. The proteins, known as chaperons, are then free to diffuse around in the periplasm and bind another incoming molecule. Other proteins degrade large molecules such as nucleic acid and large proteins to a size that is more easily transportable. These periplasmic proteins include proteases, nucleases, and phosphatases. Additional periplasmic proteins, including beta lactamase, protect the bacterium by degrading incoming **antibiotics** before they can penetrate to the cytoplasm and their site of lethal action.

The periplasm thus represents a **buffer** between the external environment and the inside of the bacterium. Gram-positive bacteria, which do not have a periplasm, excrete degradative **enzymes** that act beyond the cell to digest compounds into forms that can be taken up by the cell.

See also Bacterial ultrastructure; Chaperones; Porins

PERTUSSIS

Pertussis, commonly known as whooping cough, is a highly contagious disease caused by the **bacteria** *Bordatella pertussis*. It is characterized by classic paroxysms (spasms) of uncontrollable coughing, followed by a sharp intake of air which creates the characteristic "whoop" from which the name of the illness derives.

B. pertussis is uniquely a human pathogen (a disease causing agent, such as a bacteria, virus, fungus, etc.) meaning that it neither causes disease in other animals, nor survives in humans without resulting in disease. It exists worldwide as a disease-causing agent, and causes **epidemics** cyclically in all locations.

B. pertussis causes its most severe symptoms by attacking specifically those cells in the respiratory tract which have cilia. Cilia are small, hair-like projections that beat constantly, and serve to constantly sweep the respiratory tract clean of

such debris as mucus, bacteria, **viruses**, and dead cells. When *B. pertussis* interferes with this janitorial function, mucus and cellular debris accumulate and cause constant irritation to the respiratory tract, triggering the cough reflex and increasing further mucus production.

Although the disease can occur at any age, children under the age of two, particularly infants, are greatest risk. Once an individual has been exposed to *B. pertussis*, subsequent exposures result in a mild illness similar to the common **cold** and are thus usually not identifiable as resulting from *B. pertussis*.

Whooping cough has four somewhat overlapping stages: incubation, catarrhal stage, paroxysmal stage, and convalescent stage.

An individual usually acquires *B. pertussis* by inhaling droplets infected with the bacteria, coughed into the air by an individual already suffering from whooping cough symptoms. Incubation occurs during a week to two week period following exposure to *B. pertussis*. During the incubation period, the bacteria penetrate the lining tissues of the entire respiratory tract.

The catarrhal stage is often mistaken for an exceedingly heavy cold. The patient has teary eyes, sneezing, fatigue, poor appetite, and a very runny nose. This stage lasts about eight days to two weeks.

The paroxysmal stage, lasting two to four weeks, is heralded by the development of the characteristic whooping cough. Spasms of uncontrollable coughing, the "whooping" sound of the sharp inspiration of air, and vomiting are hallmarks of this stage. The whoop is believed to occur due to **inflammation** and mucous which narrow the breathing tubes, causing the patient to struggle to get air in, and resulting in intense exhaustion. The paroxysms can be caused by over activity, feeding, crying, or even overhearing someone else cough.

The mucus that is produced during the paroxysmal stage is thicker and more difficult to clear than the waterier mucus of the catarrhal stage, and the patient becomes increasingly exhausted while attempting to cough clear the respiratory tract. Severely ill children may have great difficulty maintaining the normal level of oxygen in their systems, and may appear somewhat blue after a paroxysm of coughing due to the low oxygen content of their blood. Such children may also suffer from encephalopathy, a swelling and degeneration of the brain which is believed to be caused both by lack of oxygen to the brain during paroxysms, and also by bleeding into the brain caused by increased pressure during coughing. Seizures may result from decreased oxygen to the brain. Some children have such greatly increased abdominal pressure during coughing, that hernias result (hernias are the abnormal protrusion of a loop of intestine through a weaker area of muscle). Another complicating factor during this phase is the development of **pneumonia** from infection with another bacterial agent, which takes hold due to the patient's weakened condition.

If the patient survives the paroxysmal stage, recovery occurs gradually during the convalescent stage, and takes about three to four weeks. Spasms of coughing may continue to occur over a period of months, especially when a patient contracts a cold or any other respiratory infection.

By itself, pertussis is rarely fatal. Children who die of pertussis infection usually have other conditions (e.g., pneumonia, metabolic abnormalities, other infections, etc.) that complicate their illness.

The presence of a pertussis-like cough along with an increase of certain specific white blood cells (lymphocytes) is suggestive of *B. pertussis* infection, although it could occur with other pertussis-like viruses. The most accurate method of diagnosis is to **culture** (grow on a laboratory plate) the organisms obtained from swabbing mucus out of the nasopharynx (the breathing tube continuous with the nose). *B. pertussis* can then be identified during microscopic examination of the culture.

In addition to the treatment of symptoms, Treatment with the antibiotic erythromycin is helpful against *B. pertussis* infection only at very early stages of whooping cough: during incubation and early in the catarrhal stage. After the cilia, and the cells bearing those cilia, are damaged, the process cannot be reversed. Such a patient will experience the full progression of whooping cough symptoms, which will only abate when the old, damaged lining cells of the respiratory tract are replaced over time with new, healthy, cilia-bearing cells. However, treatment with erythromycin is still recommended to decrease the likelihood of *B. pertussis* spreading. In fact, it is not uncommon that all members of the household in which a patient with whooping cough lives are treated with erythromycin to prevent spread of *B. pertussis* throughout the community.

The mainstay of prevention lies in the mass **immunization** program that begins, in the United States, when an infant is two months old. The pertussis **vaccine**, most often given as one immunization together with **diphtheria** and **tetanus**, has greatly reduced the incidence of whooping cough. Unfortunately, there has been some concern about serious neurologic side effects from the vaccine itself. This concern led huge numbers of parents in England, Japan, and Sweden to avoid immunizing their children, which in turn led to epidemics of disease in those countries. Multiple carefully constructed research studies, however, have provided evidence that pertussis vaccine was not the cause of neurologic damage.

See also Bacteria and bacterial infection; History of public health; Infection and resistance; Public health, current issues; Vaccination

PETRI DISH • *see* GROWTH AND GROWTH MEDIA

PETRI, RICHARD JULIUS (1852-1921)

German physician and bacteriologist

Richard Julius Petri's prominence in the microbiology community is due primarily to his invention of the growth container that bears his name. The Petri dish has allowed the growth of **bacteria** on solid surfaces under sterile conditions.

Petri was born in the German city of Barmen. Following his elementary and high school education he embarked on training as a physician. He was enrolled at the Kaiser

Wilhelm-Akademie for military physicians from 1871 to 1875. He then undertook doctoral training as a subordinate physician at the Berlin Charité. He received his doctorate in medicine in 1876.

From 1876 until 1882 Petri practiced as a military physician. Also, during this period, from 1877 to 1879, he was assigned to a research facility called the Kaiserliches Gesundheitsamt. There, he served as the laboratory assistant to **Robert Koch**. It was in Koch's laboratory that Petri acquired his interest in bacteriology. During his stay in Koch's laboratory, under Koch's direction, Petri devised the shallow, cylindrical, covered **culture** dish now known as the Petri dish or Petri plate.

Prior to this invention, bacteria were cultured in liquid broth. But Koch foresaw the benefits of a solid slab of medium as a means of obtaining isolated colonies on the surface. In an effort to devise a solid medium, Koch experimented with slabs of gelatin positioned on glass or inside bottles. Petri realized that Koch's idea could be realized by pouring molten **agar** into the bottom of a dish and then covering the agar with an easily removable lid.

While in Koch's laboratory, Petri also developed a technique for **cloning** (or producing exact copies) of bacterial strains on slants of agar formed in test tubes, followed by subculturing of the growth onto the Petri dish. This technique is still used to this day.

Petri's involvement in bacteriology continued after leaving Koch's laboratory. From 1882 until 1885 he ran the Göbersdorf sanatorium for **tuberculosis** patients. In 1886 he assumed the direction of the Museum of **Hygiene** in Berlin, and in 1889 he returned to the Kaiserliches Gesundheitsamt as a director.

In addition to his inventions and innovations, Petri published almost 150 papers on hygiene and bacteriology.

Petri died in the German city of Zeitz.

See also Bacterial growth and division; Growth and growth media; Laboratory techniques in microbiology

Oil spill from a damaged vessel (in this case, the Japanese training ship *Ehime Maru* after it was rammed by the American military submarine USS *Greeneville* near Hawaii).

PETROLEUM MICROBIOLOGY

Petroleum microbiology is a branch of microbiology that is concerned with the activity of **microorganisms** in the formation, recovery, and uses of petroleum. Petroleum is broadly considered to encompass both oil and natural gas. The microorganisms of concern are **bacteria** and **fungi**.

Much of the experimental underpinnings of petroleum microbiology are a result of the pioneering work of Claude ZoBell. Beginning in the 1930s and extending through the late 1970s, ZoBell's research established that bacteria are important in a number of petroleum related processes.

Bacterial degradation can consume organic compounds in the ground, which is a prerequisite to the formation of petroleum.

Some bacteria can be used to improve the recovery of petroleum. For example, experiments have shown that starved bacteria, which become very small, can be pumped down into an oil field, and then resuscitated. The resuscitated bacteria plug up the very porous areas of the oil field. When water is subsequently pumped down into the field, the water will be forced to penetrate into less porous areas, and can push oil from those regions out into spaces where the oil can be pumped to the surface.

Alternatively, the flow of oil can be promoted by the use of chemicals that are known as surfactants. A variety of bacteria produce surfactants, which act to reduce the surface tension of oil-water mixtures, leading to the easier movement of the more viscous oil portion.

In a reverse application, extra-bacterial polymers, such as **glycocalyx** and xanthan gum, have been used to make water more gel-like. When this gel is injected down into an oil formation, the gel pushes the oil ahead of it.

A third area of bacterial involvement involves the modification of petroleum hydrocarbons, either before or after collection of the petroleum. Finally, bacteria have proved very

useful in the remediation of sites that are contaminated with petroleum or petroleum by-products.

The **bioremediation** aspect of petroleum microbiology has grown in importance in the latter decades of the twentieth century. In the 1980s, the massive spill of unprocessed (crude) oil off the coast of Alaska from the tanker *Exxon Valdez* demonstrated the usefulness of bacteria in the degradation of oil that was contaminating both seawater and land. Since then, researchers have identified many species of bacteria and fungi that are capable of utilizing the hydrocarbon compounds that comprise oil. The hydrocarbons can be broken down by bacteria to yield carbon dioxide and water. Furthermore, the bacteria often act as a consortium, with the degradation waste products generated by one microorganism being used as a food source by another bacterium, and so on.

A vibrant industry has been spawned around the use of bacteria as petroleum remediation agents and enhancers of oil recovery. The use of bacteria involves more than just applying an unspecified bacterial population to the spill or the oil field. Rather, the bacterial population that will be effective depends on factors, including the nature of the contaminant, **pH**, temperature, and even the size of the spaces between the rocks (i.e., permeability) in the oil field.

Not all petroleum microbiology is concerned with the beneficial aspects of microorganisms. Bacteria such as *Desulfovibrio hydrocarbonoclasticus* utilize sulfate in the generation of energy. While originally proposed as a means of improving the recovery of oil, the activity of such sulfate reducing bacteria (SRBs) actually causes the formation of acidic compounds that "sour" the petroleum formation. SRBs can also contribute to dissolution of pipeline linings that lead to the burst pipelines, and plug the spaces in the rock through which the oil normally would flow on its way to the surface. The growth of bacteria in oil pipelines is such as problem that the lines must regularly scoured clean in a process that is termed "pigging," in order to prevent pipeline blowouts. Indeed, the formation of acid-generating adherent populations of bacteria has been shown to be capable of dissolving through a steel pipeline up to 0.5 in (1.3 cm) thick within a year.

See also Biodegradable substances; Economic uses and benefits of microorganisms

Pfeiffer, Richard Friedrich Johannes (1858-1945)

German physician

Richard Pfeiffer conducted fundamental research on many aspects of bacteriology, most notably bacteriolysis ("Pfeiffer's phenomenon"), which is the destruction of **bacteria** by dissolution, usually following the introduction of sera, specific antibodies, or hypotonic solutions into host animals.

Pfeiffer was born on March 27, 1858, to a German family in the Polish town of Zduny, Poznania, a province then governed by Prussia and later by Germany as Posen, but after World War II again by Poland as Ksiestwo Poznanskie. After studying medicine at the Kaiser Wilhelm Academy in Berlin from 1875 to 1879, he served Germany as an army physician and surgeon from 1879 to 1889. He received his M.D. at Berlin in 1880, taught bacteriology at Wiesbaden, Germany, from 1884 to 1887, then returned to Berlin to become the assistant of **Robert Koch** (1843–1910) at the Institute of **Hygiene** from 1887 to 1891. Upon earning his habilitation (roughly the equivalent of a Ph.D.) in bacteriology and hygiene at Berlin in 1891, he became head of the Scientific Department of the Institute for Infectious Diseases and three years later was promoted to full professor.

Pfeiffer accompanied Koch to India in 1897 to study **bubonic plague** and to Italy in 1898 to study cholera. He moved from Berlin to Königsberg, East Prussia (now Kaliningrad, Russia) in 1899 to become professor of hygiene at that city's university. He held the same position at the University of Breslau, Silesia, (now Wroclaw, Poland) from 1909 until his retirement in 1926, when he was succeeded by his friend Carl Prausnitz (1876–1963), a pioneer in the field of clinical allergy.

While serving the German army in World War I as a hygiene inspector on the Western front, Pfeiffer achieved the rank of general, won the Iron Cross, and personally intervened to save the lives of captured French microbiologists Lèon Charles Albert Calmette (1863–1933) and Camille Guèrin (1872–1961), co-inventors of the BCG (bacille biliè de Calmette-Guèrin) **vaccine** against **tuberculosis**.

Pfeiffer discovered many essential bacteriological facts, mostly in the 1890s. Several processes, phenomena, organisms, and items of equipment are named after him. A Petri dish of **agar** with a small quantity of blood smeared across the surface is called "Pfeiffer's agar." In 1891, he discovered a genus of bacteria, *Pfeifferella*, which has since been reclassified within the genus *Pseudomonas*. In 1892 he discovered and named *Haemophilus influenzae*, sometimes called "Pfeiffer's bacillus," which he incorrectly believed to be the cause of **influenza**. It does create some respiratory infections, as well as **meningitis** and conjunctivitis, but in the 1930s, other scientists learned that influenza is actually a caused by a virus.

Collaborating with Vasily Isayevich Isayev (1854–1911), he reported in 1894 and 1895 what became known as "Pfeiffer's phenomenon," **immunization** against cholera due to bacteriolysis, the dissolution of bacteria, by the injection of serum from an immune animal. In 1894, he noticed that a certain heat-resistant toxic substance was released into solution from the cell wall of *Vibrio cholerae* only after the cell had disintegrated. Following this observation he coined the term "endotoxin" to refer to potentially toxic polysaccharide or phospholipid macromolecules that form an integral part of the cell wall of Gram-negative bacteria. In 1895, he observed bactericidal substances in the blood and named them *Antikörper* ("antibodies").

Pfeiffer died on September 15, 1945 in the German-Silesian resort city of Bad Landeck, which, after the Potsdam Conference of July 17 to August 2, 1945, became Ladek Zdroj, Poland.

See also Antibody and antigen; Antibody formation and kinetics; Bacteria and bacterial infection; Bactericidal, bacteriostatic; Bubonic plague; Epidemics, bacterial; Infection and resistance; Meningitis, bacterial and viral; Pseudomonas; Serology; Typhoid fever; Typhus

pH

The term pH refers to the concentration of hydrogen ions (H+) in a solution. An acidic environment is enriched in hydrogen ions, whereas a basic environment is relatively depleted of hydrogen ions. The pH of biological systems is an important factor that determines which microorganism is able to survive and operate in the particular environment. While most **microorganisms** prefer pH's that approximate that of distilled water, some **bacteria** thrive in environments that are extremely acidic.

The hydrogen ion concentration can be determined empirically and expressed as the pH. The pH scale ranges from 0 to 14, with 1 being the most acidic and 14 being the most basic. The pH scale is a logarithmic scale. That is, each division is different from the adjacent divisions by a factor of ten. For example, a solution that has a pH of 5 is 10 times as acidic as a solution with a pH of 6.

The range of the 14-point pH scale is enormous. Distilled water has a pH of 7. A pH of 0 corresponds to 10 million more hydrogen ions per unit volume, and is the pH of battery acid. A pH of 14 corresponds to one ten-millionth as many hydrogen ions per unit volume, compared to distilled water, and is the pH of liquid drain cleaner.

Compounds that contribute hydrogen ions to a solution are called acids. For example, hydrochloric acid (HCl) is a strong acid. This means that the compounds dissociates easily in solution to produce the ions that comprise the compound (H^+ and Cl^-). The hydrogen ion is also a proton. The more protons there are in a solution, the greater the acidity of the solution, and the lower the pH.

Mathematically, pH is calculated as the negative logarithm of the hydrogen ion concentration. For example, the hydrogen ion concentration of distilled water is 10^{-7} and hence pure water has a pH of 7.

The pH of microbiological growth media is important in ensuring that growth of the target microbes occurs. As well, keeping the pH near the starting pH is also important, because if the pH varies too widely the growth of the microorganism can be halted. This growth inhibition is due to a numbers of reasons, such as the change in shape of proteins due to the presence of more hydrogen ions. If the altered protein ceases to perform a vital function, the survival of the microorganism can be threatened. The pH of growth media is kept relatively constant by the inclusion of compounds that can absorb excess hydrogen or hydroxyl ions. Another means of maintaining pH is by the periodic addition of acid or base in the amount needed to bring the pH back to the desired value. This is usually done in conjunction with the monitoring of the solution, and is a feature of large-scale microbial growth processes, such as used in a brewery.

Microorganisms can tolerate a spectrum of pHs. However, an individual microbe usually has an internal pH that is close to that of distilled water. The surrounding cell membranes and external layers such as the **glycocalyx** contribute to buffering the cell from the different pH of the surrounding environment.

Some microorganisms are capable of modifying the pH of their environment. For example, bacteria that utilize the sugar glucose can produce lactic acid, which can lower the pH of the environment by up to two pH units. Another example is that of **yeast**. These microorganisms can actively pump hydrogen ions out of the cell into the environment, creating more acidic conditions. Acidic conditions can also result from the microbial utilization of a basic compound such as ammonia. Conversely, some microorganisms can raise the pH by the release of ammonia.

The ability of microbes to acidify the environment has been long exploited in the pickling process. Foods commonly pickled include cucumbers, cabbage (i.e., sauerkraut), milk (i.e., buttermilk), and some meats. As well, the production of vinegar relies upon the pH decrease caused by the bacterial production of acetic acid.

See also Biochemistry; Buffer; Extremophiles

Phage genetics

Bacteriophages, **viruses** that infect **bacteria**, are useful in the study of how genes function. The attributes of bacteriophages include their small size and simplicity of genetic organization.

The most intensively studied **bacteriophage** is the phage called lambda. It is an important model system for the latent infection of mammalian cells by **retroviruses**, and it has been widely used for **cloning** purposes. Lambda is the prototype of a group of phages that are able to infect a cell and redirect the cell to become a factory for the production of new virus particles. This process ultimately results in the destruction of the host cell (lysis). This process is called the lytic cycle. On the other hand, lambda can infect a cell, direct the integration of its genome into the **DNA** of the host, and then reside there. Each time the host genome replicates, the viral genome undergoes replication, until such time as it activates and produces new virus particles and lysis occurs. This process is called the lysogenic cycle.

Lambda and other phages, which can establish lytic or lysogenic cycles, are called temperate phages. Other examples of temperate phages are bacteriophage mu and P1. Mu inserts randomly into the host chromosome causing insertional **mutations** where intergrations take place. The P1 genome exists in the host cell as an autonomous, self-replicating plasmid.

Phage **gene** expression during the lytic and lysogenic cycles uses the host **RNA** polymerase, as do other viruses. However, lambda is unique in using a type of regulation called antitermination.

As host RNA polymerase transcribes the lambda genome, two proteins are produced. They are called cro (for "control of repressor and other things") and N. If the lytic

pathway is followed, **transcription** of the remainder of the viral genes occurs, and assembly of the virus particles will occur. The N protein functions in this process, ensuring that transcription does not terminate.

The path to **lysogeny** occurs differently, involving a protein called cI. The protein is a repressor and its function is to bind to operator sequences and prevent transcription. Expression of cI will induce the phage genome to integrate into the host genome. When integrated, only the cI will be produced, so as to maintain the lysogenic state.

The virus adopts the lytic or lysogenic path early following infection of the host bacterium. The fate of the viral genetic material is governed by a competition between the cro and cI proteins. Both can bind to the same operator region. The region has three binding zones—cro and cI occupy these zones in reverse order. The protein, which is able to occupy the preferred regions of the operator first, stimulates its further synthesis and blocks synthesis of the other protein.

Analysis of the genetics of phage activity is routinely accomplished using a **plaque** assay. When a phage infects a lawn or layer of bacterial cells growing on a flat surface, a clear zone of lysis can occur. The clear area is called a plaque.

Aside from their utility in the study of gene expression, phage genetics has been put to practical use as well. Cloning of the human insulin gene in bacteria was accomplished using a bacteriophage as a vector. The phage delivered to the bacterium a recombinant plasmid containing the insulin gene. M13, a single-stranded filamentous DNA bacteriophage, has long been used as a cloning vehicle for **molecular biology**. It is also valuable for use in DNA sequencing, because the viral particle contains single-stranded DNA, which is an ideal template for sequencing. T7 phage, which infects *Escherichia coli,* and some strains of *Shigella* and *Pasteurella,* is a popular vehicle for cloning of complimentary DNA. Also, the T7 promoter and RNA polymerase are in widespread use as a system for regulatable or high-level gene expression.

See also Bacteriophage and bacteriophage typing; Microbial genetics; Viral genetics

Phage therapy

Bacteriophage are well suited to deliver therapeutic payloads (i.e., deliver specific genes into a host organism). Characteristic of **viruses**, they require a host in which to make copies of their genetic material, and to assemble progeny virus particles. Bacteriophage are more specific in that they infect solely **bacteria**.

The use of phage to treat bacterial infections was popular early in the twentieth century, prior to the mainstream use of **antibiotics**. Doctors used phages as treatment for illnesses ranging from cholera to typhoid fevers. Sometimes, phage-containing liquid was poured into the wound. Oral, aerosol, and injection administrations were also used. With the advent of antibiotic therapy, the use of phage was abandoned. But now, the increasing resistance of bacteria to antibiotics has sparked a reassessment of phage therapy.

Lytic bacteriophage, which destroy the bacterial cell as part of their infectious process, are used in therapy. Much of the focus in the past 15 years has been on nosocomial, or hospital-acquired infections, where multi-drug-resistant organisms have become a particularly lethal problem.

Bacteriophage offer several advantages as therapeutic agents. Their target specificity causes less disruption to the normal host bacterial flora, some species of which are vital in maintaining the ecological balance in various areas of the body, than does the administration of a relatively less specific antibiotic. Few side effects are evident with phage therapy, particularly allergic reactions, such as can occur to some antibiotics. Large numbers of phage can be prepared easily and inexpensively. Finally, for localized uses, phage have the special advantage that they can continue multiplying and penetrating deeper as long as the infection is present, rather than decreasing rapidly in concentration below the surface like antibiotics.

In addition to their specific lethal activity against target bacteria, the relatively new field of **gene** therapy has also utilized phage. Recombinant phage, in which carry a bit of non-viral genetic material has been incorporated into their genome, can deliver the recombinant **DNA** or **RNA** to the recipient genome. The prime use of this strategy to date has been the replacement of a defective or deleterious host gene with the copy carried by the phage. Presently, however, technical safety issues and ethical considerations have limited the potential of phage genetic therapy.

See also Bacteriophage and bacteriophage typing; Microbial genetics; Viral genetics; Viral vectors in gene therapy

Phagocyte and phagocytosis

In the late 1800s and early 1900s, scientific researchers worked to uncover the mysteries of the body's immune system—the ways in which the body protects itself against harmful invading substances. One line of investigation showed that **immunity** is due to protective substances in the blood—antibodies—that act on disease organisms or toxins.

An additional discovery was made by the Russian-French microbiologist **Élie Metchnikoff** (1845–1916) in the 1880s. While studying transparent starfish larvae, Metchnikoff observed certain cells move to, surround, and engulf foreign particles introduced into the larvae. Metchnikoff then observed the same phenomenon in water fleas. Studying more complicated animals, Metchnikoff found similar cells moving freely in the blood and tissues. He was able to show that these mobile cells—the white blood corpuscles—in higher animals as well as humans also ingested **bacteria**.

The white blood cells responded to the site of an infection and engulfed and destroyed the invading bacteria. Metchnikoff called these bacteria-ingesting cells phagocytes, Greek for "eating cells," and published his findings in 1883.

The process of digestion by phagocytes is termed phagocytosis.

In 1905, English pathologist Almroth Wright (1861–1947) demonstrated that phagocytosis and **antibody** factors in the blood worked together in the immune response process.

See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Antibody, monoclonal; Antigenic mimicry; Immune system; Immunity, active, passive, and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunization; Immunogenetics; Immunology; Infection and resistance; Inflammation

PHAGOCYTE DEFECTS • *see* IMMUNODEFICIENCY DISEASE SYNDROMES

PHENOTYPE AND PHENOTYPIC VARIATION

The word **phenotype** refers to the observable characters or attributes of individual organisms, including their morphology, physiology, behavior, and other traits. The phenotype of an organism is limited by the boundaries of its specific genetic **complement** (**genotype**), but is also influenced by environmental factors that impact the expression of genetic potential.

All organisms have unique genetic information, which is embodied in the particular nucleotide sequences of their **DNA** (**deoxyribonucleic acid**), the genetic biochemical of almost all organisms, except for **viruses** and **bacteria** that utilize **RNA** as their genetic material. The genotype is fixed within an individual organism but is subject to change (**mutations**) from one generation to the next due to low rates of natural or spontaneous mutation. However, there is a certain degree of developmental flexibility in the phenotype, which is the actual or outward expression of the genetic information in terms of anatomy, behavior, and **biochemistry**. This flexibility can occur because the expression of genetic potential is affected by environmental conditions and other circumstances.

Consider, for example, genetically identical bacterial cells, with a fixed complement of genetic each plated on different gels. If one bacterium is colonized under ideal conditions, it can grow and colonize its full genetic potential. However, if a genetically identical bacterium is exposed to improper nutrients or is otherwise grown under adverse conditions, colony formation may be stunted. Such varying growth patterns of the same genotype are referred to as phenotypic plasticity. Some traits of organisms, however, are fixed genetically, and their expression is not affected by environmental conditions. Moreover, the ability of species to exhibit phenotypically plastic responses to environmental variations is itself, to a substantial degree, genetically determined. Therefore, phenotypic plasticity reflects both genetic capability and varying expression of that capability, depending on circumstances.

Phenotypic variation is essential for **evolution**. Without a discernable difference among individuals in a population there are no genetic **selection** pressures acting to alter the variety and types of alleles (forms of genes) present in a population. Accordingly, genetic mutations that do not result in phenotypic change are essentially masked from evolutionary mechanisms.

Phenetic similarity results when phenotypic differences among individuals are slight. In such cases, it may take a significant alteration in environmental conditions to produce significant selection pressure that results in more dramatic phenotypic differences. Phenotypic differences lead to differences in fitness and affect adaptation.

See also DNA (Deoxyribonucleic acid); Molecular biology and molecular genetics

PHENOTYPE • *see* GENOTYPE AND PHENOTYPE

PHOSPHOLIPIDS

Phospholipids are complex lipids made up of fatty acids, alcohols, and phosphate. They are extremely important components of living cells, with both structural and metabolic roles. They are the chief constituents of most biological membranes.

At one end of a phospholipid molecule is a phosphate group linked to an alcohol. This is a polar part of the molecule—it has an electric charge and is water-soluble (hydrophilic). At the other end of the molecule are fatty acids, which are non-polar, **hydrophobic**, fat soluble, and water insoluble.

Because of the dual nature of the phospholipid molecules, with a water-soluble group attached to a water-insoluble group in the same molecule, they are called amphipathic or polar lipids. The amphipathic nature of phospholipids make them ideal components of biological membranes, where they form a lipid bilayer with the polar region of each layer facing out to interact with water, and the non-polar fatty acid "tail" portions pointing inward toward each other in the interior of the bilayer. The lipid bilayer structure of cell membranes makes them nearly impermeable to polar molecules such as ions, but proteins embedded in the membrane are able to carry many substances through that they could not otherwise pass.

Phosphoglycerides, considered by some as synonymous for phospholipids, are structurally related to 3-phosphoglyceraldehyde (PGA), an intermediate in the catabolic **metabolism** of glucose. Phosphoglycerides differ from phospholipids because they contain an alcohol rather than an aldehyde group on the 1-carbon. Fatty acids are attached by an ester linkage to one or both of the free hydroxyl (-OH) groups of the glyceride on carbons 1 and 2. Except in phosphatidic acid, the simplest of all phosphoglycerides, the phosphate attached to the 3-carbon of the glyceride is also linked to another alcohol. The nature of this alcohol varies considerably.

See also Bacteremic; Bacterial growth and division; Bacterial membranes and cell wall; Bacterial surface layers; Bacterial ultrastructure; Biochemistry; Cell membrane transport; Membrane fluidity

PHOTOSYNTHESIS

Photosynthesis is the biological conversion of light energy into chemical energy. This occurs in green plants, algae, and photosynthetic **bacteria**.

Much of the early knowledge of bacterial photosynthesis came from the work of Dutch-born microbiologist **Cornelius van Neil** (1897–1985). During his career at the Marine Research Station in Monterey, California, van Neil studied photosynthesis in anaerobic bacteria. Like higher plants, these bacteria manufacture carbohydrates during photosynthesis. But, unlike plants, they do not produce oxygen during the photosynthetic process. Furthermore, the bacteria use a compound called bacteriochlorophyll rather than **chlorophyll** as a photosynthetic pigment. Van Neil found that all species of photosynthetic bacteria require a compound that the bacteria can oxidize (i.e., remove an electron from). For example, the purple sulfur bacteria use hydrogen sulfide.

Since van Neil's time, the structure of the photosynthetic apparatus has been deduced. The study of photosynthesis is currently an active area of research in biology. Crystals of the photosynthetic reaction center from the anaerobic photosynthetic bacterium *Rhodopseudomonas viridis* were created in the 1980s by Hartmut Michel and Johann Deisenhofer, who then used x-ray crystallography to determine the three-dimensional structure of the photosynthetic protein. In 1988, the two scientists shared the Nobel Prize in Chemistry with Robert Huber for this research.

Photosynthesis consists of two series of biochemical reactions, called the light reactions and the dark reactions. The light reactions use the light energy absorbed by chlorophyll to synthesize structurally unstable high-energy molecules. The dark reactions use these high-energy molecules to manufacture carbohydrates. The carbohydrates are stable structures that can be stored by plants and by bacteria. Although the dark reactions do not require light, they often occur in the light because they are dependent upon the light reactions. In higher plants and algae, the light and dark reactions of photosynthesis occur in chloroplasts, specialized chlorophyll-containing intracellular structures that are enclosed by double membranes.

In the light reactions of photosynthesis, light energy excites photosynthetic pigments to higher energy levels and this energy is used to make two high energy compounds, ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate). ATP and NADPH are consumed during the subsequent dark reactions in the synthesis of carbohydrates.

In algae, the light reactions occur on the so-called thylakoid membranes of the chloroplasts. The thylakoid membranes are inner membranes of the chloroplasts. These membranes are arranged like flattened sacs. The thylakoids are often stacked on top of one another, like a roll of coins. Such a stack is referred to as a granum. ATP can also be made by a special series of light reactions, referred to as cyclic photophosphorylation, which occurs in the thylakoid membranes of the **chloroplast**.

Algae are capable of photosynthetic generation of energy. There are many different groups of photosynthetic algae. Like higher plants, they all have chlorophyll-a as a photosynthetic pigment, two photosystems (PS-I and PS-II), and the same overall chemical reactions for photosynthesis. Algae differ from higher plants in having different complements of additional chlorophylls. *Chlorophyta* and *Euglenophyta* have chlorophyll-a and chlorophyll-b. *Chrysophyta*, *Pyrrophyta*, and *Phaeophyta* have chlorophyll-a and chlorophyll-c. *Rhodophyta* have chlorophyll-a and chlorophyll-d. The different chlorophylls and other photosynthetic pigments allow algae to utilize different regions of the solar spectrum to drive photosynthesis.

A number of photosynthetic bacteria are known. One example are the bacteria of the genus *Cyanobacteria*. These bacteria were formerly called the **blue-green algae** and were once considered members of the plant kingdom. However, unlike the true algae, cyanobacteria are prokaryotes, in that their **DNA** is not sequestered within a **nucleus**. Like higher plants, they have chlorophyll-a as a photosynthetic pigment, two photosystems (PS-I and PS-II), and the same overall equation for photosynthesis (equation 1). Cyanobacteria differ from higher plants in that they have additional photosynthetic pigments, referred to as phycobilins. Phycobilins absorb different wavelengths of light than chlorophyll and thus increase the wavelength range, which can drive photosynthesis. Phycobilins are also present in the Rhodophyte algae, suggesting a possible evolutionary relationship between these two groups.

Cyanobacteria are the predominant photosynthetic organism in anaerobic fresh and marine water.

Another photosynthetic bacterial group is called cloroxybacteria. This group is represented by a single genus called *Prochloron*. Like higher plants, *Prochloron* has chlorophyll-a, chlorophyll-b, and carotenoids as photosynthetic pigments, two photosystems (PS-I and PS-II), and the same overall equation for photosynthesis. *Prochloron* is rather like a free-living chloroplast from a higher plant.

Another group of photosynthetic bacteria are known as the purple non-sulfur bacteria (e.g., *Rhodospirillum rubrum*. The bacteria contain bacteriochlorophyll a or b positioned on specialized membranes that are extensions of the cytoplasmic membrane.

Anaerobic photosynthetic bacteria is a group of bacteria that do not produce oxygen during photosynthesis and only photosynthesize in environments that are devoid of oxygen. These bacteria use carbon dioxide and a substrate such as hydrogen sulfide to make carbohydrates. They have bacteriochlorophylls and other photosynthetic pigments that are similar to the chlorophylls used by higher plants. But, in contrast to higher plants, algae and cyanobacteria, the anaerobic photosynthetic bacteria have just one photosystem that is similar to PS-I. These bacteria likely represent a very ancient photosynthetic microbe.

The final photosynthetic bacteria are in the genus *Halobacterium*. Halobacteria thrive in very salty environments, such as the Dead Sea and the Great Salt Lake. Halobacteria are unique in that they perform photosynthesis without chlorophyll. Instead, their photosynthetic pigments are bacteriorhodopsin and halorhodopsin. These pigments are similar to sensory rhodopsin, the pigment used by humans and

other animals for vision. Bacteriorhodopsin and halorhodopsin are embedded in the cell membranes of halobacteria and each pigment consists of retinal, a vitamin-A derivative, bound to a protein. Irradiation of these pigments causes a structural change in their retinal. This is referred to as photoisomerization. Retinal photoisomerization leads to the synthesis of ATP. Halobacteria have two additional rhodopsins, sensory rhodopsin-I and sensory rhodopsin-II. These compounds regulate phototaxis, the directional movement in response to light.

See also Evolutionary origin of bacteria and viruses

PHOTOSYNTHETIC MICROORGANISMS

Life first evolved in the primordial oceans of Earth approximately four billion years ago. The first life forms were prokaryotes, or non-nucleated unicellular organisms, which divided in two domains, the **Bacteria** and **Archaea**. They lived around hot sulfurous geological and volcanic vents on the ocean floor, forming distinct biofilms, organized in multilayered symbiotic communities, known as microbial mats. Fossil evidence suggests that these first communities were not photosynthetic, i.e., did not use the energy of light to convert carbon dioxide and water into glucose, releasing oxygen in the process. About 3.7 billions years ago, anoxygenic photosynthetic **microorganisms** probably appeared on top of pre-photosynthetic biofilms formed by bacterial and Archaean sulphate-processers. Anoxygenic photosynthesizers use electrons donated by sulphur, hydrogen sulfide, hydrogen, and a variety of organic chemicals released by other bacteria and Archaea. This ancestor species, known as protochlorophylls, did not synthesized **chlorophyll** and did not release oxygen during **photosynthesis**. Moreover, in that deep-water environment, they probably used infrared thermo taxis rather than sunlight as a source of energy.

Protochlorophylls are assumed to be the common ancestors of two evolutionary branches of oxygenic photosynthetic organisms that began evolving around 2.8 billion years ago: the bacteriochlorophyll and the chlorophylls. Bacteriochlorophyll gave origin to chloroflexus, sulfur green bacteria, sulfur purple bacteria, non-sulfur purple bacteria, and finally to oxygen-respiring bacteria. Chlorophylls originated Cyanobacteria, from which chloroplasts such as red algae, cryptomonads, **dinoflagellates**, crysophytes, brown algae, euglenoids, and finally green plants evolved. The first convincing paleontological evidence of eukaryotic microfossils (chloroplasts) was dated 1.5 at billion years old. In oxygenic photosynthesis, electrons are donated by water molecules and the energy source is the visible spectrum of visible light. However, the chemical elements utilized by oxygenic photosynthetic organisms to capture electrons divide them in two families, the Photosystem I Family and the Photosystem II Family. Photosystem II organisms, such as *Chloroflexus aurantiacus* (an ancient green bacterium) and sulfur purple bacteria, use pigments and quinones as electron acceptors, whereas member of the Photosystem I Family, such as green sulfur bacteria, Cyanobacteria, and chloroplasts use iron-sulphur centers as electron acceptors.

It is generally accepted that the **evolution** of oxygenic photosynthetic microorganisms was a crucial step for the increase of atmospheric oxygen levels and the subsequent burst of biological evolution of new aerobic species. About 3.5 billion years ago, the planet atmosphere was poor in oxygen and abundant in carbon dioxide and sulfuric gases, due to intense volcanic activity. This atmosphere favored the evolution of chemotrophic Bacteria and Archaea. As the populations of oxygenic photosynthetic microorganisms gradually expanded, they started increasing the atmospheric oxygen level two billion years ago, stabilizing it at its present level of 20% about 1.5 billion years ago, and additionally, reduced the carbon dioxide levels in the process. Microbial photosynthetic activity increased the planetary biological productivity by a factor of 100–1,000, opening new pathways of biological evolution and leading to biogeochemical changes that allowed life to evolve and colonize new environmental niches. The new atmospheric and biogeochemical conditions created by photosynthetic microorganisms allowed the subsequent appearance of plants about 1.2 billion years ago, and 600 million years later, the evolution of the first vertebrates, followed 70 million years later by the Cambrian burst of biological diversity.

See also Aerobes; Autotrophic bacteria; Biofilm formation and dynamic behavior; Biogeochemical cycles; Carbon cycle in microorganisms; Chemoautotrophic and chemolithotrophic bacteria; Electron transport system; Evolutionary origin of bacteria and viruses; Fossilization of bacteria; Hydrothermal vents; Plankton and planktonic bacteria; Sulfur cycle in microorganisms

PHYLOGENY

Phylogeny is the inferred evolutionary history of a group of organisms (including **microorganisms**). Paleontologists are interested in understanding life through time, not just at one time in the past or present, but over long periods of past time. Before they can attempt to reconstruct the forms, functions, and lives of once-living organisms, paleontologists have to place these organisms in context. The relationships of those organisms to each other are based on the ways they have branched out, or diverged, from a common ancestor. A phylogeny is usually represented as a phylogenetic tree or cladogram, which are like genealogies of species.

Phylogenetics, the science of phylogeny, is one part of the larger field of systematics, which also includes taxonomy. Taxonomy is the science of naming and classifying the diversity of organisms. Not only is phylogeny important for understanding paleontology (study of fossils), however, paleontology in turn contributes to phylogeny. Many groups of organisms are now extinct, and without their fossils we would not have as clear a picture of how modern life is interrelated.

There is an amazing diversity of life, both living and extinct. For scientists to communicate with each other about these many organisms, there must also be a classification of these organisms into groups. Ideally, the classification should

be based on the evolutionary history of life, such that it predicts properties of newly discovered or poorly known organisms.

Phylogenetic systematics is an attempt to understand the evolutionary interrelationships of living things, trying to interpret the way in which life has diversified and changed over time. While classification is primarily the creation of names for groups, systematics goes beyond this to elucidate new theories of the mechanisms of **evolution**.

Cladistics is a particular method of hypothesizing relationships among organisms. Like other methods, it has its own set of assumptions, procedures, and limitations. Cladistics is now accepted as the best method available for phylogenetic analysis, for it provides an explicit and testable hypothesis of organismal relationships.

The basic idea behind cladistics is that members of a group share a common evolutionary history, and are "closely related," more so to members of the same group than to other organisms. These groups are recognized by sharing unique features that were not present in distant ancestors. These shared derived characteristics are called synapomorphies. Synapomorphies are the basis for cladistics.

In a cladistic analysis, one attempts to identify which organisms belong together in groups, or clades, by examining specific derived features or characters that those organisms share. For example, if a genus of **bacteria** forms a specific color or shaped **colony**, then those characters might be a useful character for determining the evolutionary relationships of other bacteria. Characters that define a clade are called synapomorphies. Characters that do not unite a clade because they are primitive are called plesiomorphies.

In a cladistic analysis, it is important to know which character states are primitive and which are derived (that is, evolved from the primitive state). A technique called outgroup comparison is commonly used to make this determination. In outgroup comparison, the individuals of interest (the ingroup) are compared with a close relative. If some of the individuals of the ingroup possess the same character state as the outgroup, then that character state is assumed to be primitive.

There are three basic assumptions in cladistics:

1. Any group of organisms are related by descent from a common ancestor.
2. There is a bifurcating pattern of cladogenesis.
3. Change in characteristics occurs in lineages over time.

The first assumption is a general assumption made for all evolutionary biology. It essentially means that life arose on Earth only once, and therefore all organisms are related in one way or another. Because of this, scientists can take any collection of organisms and determine a meaningful pattern of relationships, provided they have the right kind of information.

The second assumption is that new kinds of organisms may arise when existing species or populations divide into exactly two groups. The final assumption, that characteristics of organisms change over time, is the most important assumption in cladistics. It is only when characteristics change that different lineages or groups are recognized. The convention is to call the "original" state of the characteristic plesiomorphic and the "changed" state apomorphic. The terms *primitive* and *derived* have also been used for these states, but they are often avoided by cladists, since those terms have been abused in the past.

Cladistics is useful for creating systems of classification. It is now the most commonly used method to classify organisms because it recognizes and employs evolutionary theory. Cladistics predicts the properties of organisms. It produces hypotheses about the relationships of organisms in a way that makes it possible to predict properties of the organisms. This can be especially important in cases when particular genes or biological compounds are being sought. Such genes and compounds are being sought all the time by companies interested in improving bacterial strains, disease resistance, and in the search for medicines. Only an hypothesis based on evolutionary theory, such as cladistic hypotheses, can be used for these endeavors.

As an example, consider the plant species *Taxus brevifolia*. This species produces a compound, taxol, which is useful for treating cancer. Unfortunately, large quantities of bark from this rare tree are required to produce enough taxol for a single patient. Through cladistic analysis, a phylogeny for the genus *Taxus* has been produced that shows *Taxus cuspidata*, a common ornamental shrub, to be a very close relative of *T. brevifolia*. *Taxus cuspidata*, then, may also produce large enough quantities of taxol to be useful. Having a classification based on evolutionary descent will allow scientists to select the species most likely to produce taxol.

Cladistics helps to elucidate mechanisms of evolution. Unlike previous systems of analyzing relationships, cladistics is explicitly evolutionary. Because of this, it is possible to examine the way characters change within groups over time, the direction in which characters change, and the relative frequency with which they change. It is also possible to compare the descendants of a single ancestor and observe patterns of origin and extinction in these groups, or to look at relative size and diversity of the groups. Perhaps the most important feature of cladistics is its use in testing long-standing hypotheses about adaptation.

See also Bacterial kingdoms; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Microbial genetics; Viral genetics

PILI • *see* BACTERIAL APPENDAGES

PIPETTE

A pipette is a piece of volumetric glassware used to transfer quantitatively a desired volume of solution from one container to another. Pipettes are calibrated at a specified temperature (usually 68°F [20°C] or 77°F [25°C]) either to contain (TC) or to deliver (TD) the stated volume indicated by the etched/painted markings on the pipette side. Pipettes that are marked TD generally deliver the desired volume with free drainage; whereas in the case of pipettes marked TC the last drop must be blown out or washed out with an appropriate solvent.

Researcher dispensing sample into an analysis tray.

For high-accuracy chemical analysis and research work, a volumetric transfer pipette is preferred. Volumetric transfer pipettes are calibrated to deliver a fixed liquid volume with free drainage, and are available in sizes ranging from 0.5–200 mL. Class A pipettes with volumes greater than 5 mL have a tolerance of +/-0.2% or better. The accuracy and precision of the smaller Class A pipettes and of the Class B pipettes are less. The Ostwald-Folin pipette is similar to the volumetric transfer pipette, except that the last drop should be blown out. Mohr and serological pipettes have graduated volumetric markings, and are designed to deliver various volumes with an accuracy of +/- 0.5-1.0%. The volume of liquid transferred is the difference between the volumes indicated before and after delivery. Serological pipettes are calibrated all the way to the tip, and the last drop should be blown out. The calibration markings on Mohr pipettes, on the other hand, begins well above the tip. Lambda pipettes are used to transfer very small liquid volumes down to 1 microliter. Dropping pipettes (i.e., medicine droppers) and Pasteur pipettes are usually uncalibrated, and are used to transfer liquids only when accurate quantification is not necessary.

Automatic dispensing pipettes and micropipettes are available commercially. Automatic dispensing pipettes, in sizes ranging from 1–2,000 mL, permit fast, repetitive delivery of a given volume of solution from a dispensing bottle. Micropipettes consist of a cylinder with a thumb-operated airtight plunger. A disposable plastic tip attaches to the end of the cylinder, the plunger is depressed, and the plastic tip is immersed in the sample solution. The liquid enters the tip when the plunger is released. The solution never touches the plunger. Micropipettes generally have fixed volumes, however, some models have provisions for adjustable volume settings. Micropipettes are extremely useful in clinical and biochemical applications where errors of +/- 1% are acceptable, and where problems of **contamination** make disposable tips desirable.

See also Laboratory techniques in immunology; Laboratory techniques in microbiology

PITTMAN, MARGARET (1901-1995)

American bacteriologist

An expert in the development and standardization of bacterial vaccines, Margaret Pittman advanced the fight against such diseases as whooping cough (**pertussis**), **tetanus**, typhoid, cholera, **anthrax**, **meningitis**, and conjunctivitis.

Pittman was born on January 20, 1901 in Prairie Grove, Arkansas, the daughter of a physician, James ("Dr. Jim") Pittman, and the former Virginia Alice McCormick. The family moved to nearby Cincinnati, Arkansas, in 1909. Her father was the only doctor in that rural area, and she sometimes helped him on his rounds or with anesthesia. Her formal education was sporadic until three years of high school in Prairie Grove and two years of music seminary in Siloam Springs, Arkansas. As a member of the class of 1923 at Hendrix College, Conway, Arkansas, she double-majored in mathematics and biology, and won the Walter Edwin Hogan Mathematics Award in 1922. From 1923 until 1925 in Searcy, Arkansas, she taught and served as principal at Galloway Woman's College, which merged with Hendrix in 1933. She received her M.S. in 1926 and her Ph.D. in 1929, both in bacteriology from the University of Chicago.

Pittman's landmark article of 1931, "Variation and Type Specificity in the Bacterial Species *Haemophilus Influenzae*," showed that the pathogenicity (disease-causing quality) of this microbe is determined by minor differences in its physical nature, such as the presence or absence of a polysaccharide capsule. For all microbes, these differences can be classed as strains or types. Pittman identified six serotypes of *Haemophilus influenzae*, which she labeled "a" through "f." Serotype b (Hib) is the most pathogenic, causing meningitis and several other serious infections. Her work led to the development of polysaccharide vaccines that immunize against Hib.

Pittman conducted her bacteriological research at the Rockefeller Institute for Medical Research (later Rockefeller University) from 1928 to 1934, at the New York State Department of Health from 1934 to 1936, and from 1936 until the end of her career at the National Institutes of Health (NIH). Among the subjects of her research were tetanus, toxins and antitoxins, sera and antisera, the genus *Bordetella*, the Koch-Weeks bacillus, the standardization of vaccines, and cholera. Some of this work was done abroad under the auspices of the **World Health Organization** (**WHO**). In 1957, Pittman became the first woman director of an NIH laboratory when she was chosen chief of the Laboratory of Bacterial Products in the Division of Biologics Standards. She held that post until she retired in 1971. Thereafter she lived quietly but productively in Temple Hills, Maryland, serving occasionally as a guest researcher and consultant for NIH, the U.S. Food and Drug Administration (FDA), and WHO, and remaining active in the United Methodist Church, especially through Wesley Theological Seminary in Washington, D.C. She died on August 19, 1995.

In 1994, NIH inaugurated the Margaret Pittman Lecture Series and the American Society for Microbiology presented its first Margaret Pittman Award. On October 19, 1995, John Bennett Robbins (b. 1932) and Ronald D. Sekura, both of the National Institute of Child Health and Human Development (NICHD) published an article in the *New England Journal of Medicine*, announcing their new pertussis **vaccine**, based on Pittman's research at the FDA.

See also Antiserum and antitoxin; Bacteria and bacterial infection; Meningitis, bacterial and viral; Pneumonia, bacterial and viral; Serology; Tetanus and tetanus immunization; Typhoid fever

PLAGUE, BUBONIC • *see* BUBONIC PLAGUE

PLANKTON AND PLANKTONIC BACTERIA

Plankton and planktonic **bacteria** share two features. First, they are both single-celled creatures. Second, they live as floating organisms in the respective environments.

Plankton and planktonic bacteria are actually quite different from one another. Plankton is comprised of two main types, neither of which is bacterial. One type of plankton, the one of most relevance to this volume, is phytoplankton. Phytoplankton are plants. The second type of plankton is **zooplankton**. These are microscopic animals. Phytoplankton form the basis of the food chain in the ocean.

Phytoplankton are fundamentally important to life on Earth for several reasons. In the oceans, they are the beginning of the food chain. Existing in the oceans in huge quantities, phytoplankton are eaten by small fish and animals that are in turn consumed by larger species. Their numbers can be so huge that they are detectable using specialized satellite imaging, which is exploited by the commercial fishing industry to pinpoint likely areas in which to catch fish.

Phytoplankton, through their central role in the carbon cycle, are also a critical part of the ocean chemistry. The carbon dioxide content in the atmosphere is in balance with the content in the oceans. The photosynthetic activity of phytoplankton removes carbon dioxide from the water and releases oxygen as a by-product to the atmosphere. This allows the oceans to absorb more carbon dioxide from the air. Phytoplankton, therefore, act to keep the atmospheric level of carbon dioxide from increasing, which causes the atmosphere to heat up, and also replenish the oxygen level of the atmosphere.

When phytoplankton die and sink to the ocean floor, the carbon contained in them is lost from global circulation. This is beneficial because if the carbon from all dead matter was recycled into the atmosphere as carbon dioxide, the balance of carbon dioxide would be thrown off, and a massive atmospheric temperature increase would occur.

Phytoplankton are also being recognized as an indicator for the physical status of the oceans. They require a fairly limited range of water temperature for healthy growth. So, a downturn in phytoplankton survival can be an early indicator of changing conditions, both at a local level (such as the presence of pollutants) and at a global level (global warming).

Planktonic bacteria are free-living bacteria. They are the populations that grow in the familiar test tube and flask cultures in the microbiology laboratory. The opposite mode of growth is the adherent, or sessile, type of growth.

Planktonic bacteria have been recognized for centuries. They are some of the "animalcules" described by **Antoni van Leeuwenhoek** in 1673 using a **microscope** of his own design. Indeed, much of the knowledge of microbiology is based on work using these free-floating organisms. Research over the past two decades has shown that the planktonic mode of growth is secondary to the adherent type of growth. Additionally, the character of planktonic bacteria is very different from their adherent counterparts. Planktonic bacteria tend to have surfaces that are relatively hydrophilic (water loving), and the pattern of **gene** expression is markedly different from bacteria growing on a surface. Also, planktonic bacteria tend not to have a surrounding coat made of various sugars (this coat is also called a **glycocalyx**), and so the bacteria tend to be more susceptible to antibacterial agent such as **antibiotics**. Paradoxically, most of the knowledge of antibiotic activity has been based on experiments with planktonic bacteria.

When grown in a **culture** where no new nutrients are added, planktonic bacteria typically exhibit the four stages of population development that are known as lag phase, logarithmic (or exponential) phase, stationary phase, and death (or decline) phase. It is also possible to grow planktonic bacteria under conditions where fresh food is added at the same rate as culture is removed. Then, the bacteria will grow as fast as the rate of addition of the new food source and can remain in this state for as long as the conditions are maintained. Thus, planktonic bacteria display a great range in the speeds at which they can grow. These abilities, as well as other changes the bacteria are capable of, is possible because the bacteria are phenotypically "plastic;" that is, they are very adaptable. Their adherent counterparts tend to be less responsive to environmental change.

Planktonic bacteria are susceptible to eradication by the **immune system** of man and other animals. Examination of many infectious bacteria has demonstrated that once in a host, planktonic bacteria tend to adopt several strategies to evade the host reaction. These strategies include formation of the adherent, glycocalyx enclosed populations, the elaboration of the glycocalyx around individual bacteria, and entry into the cells of the host.

It is becoming increasingly evident that the planktonic bacteria first observed by Leeuwenhoek and which is the staple of lab studies even today is rather atypical of the state of the bacteria in nature and in infections. Thus, in a sense, the planktonic bacteria in the test tube culture is an artifact.

See also Carbon cycle in microorganisms

PLANT VIRUSES

Plant **viruses** are viruses that multiply by infecting plant cells and utilizing the plant cell's genetic replication machinery to manufacture new virus particles.

Plant viruses do not infect just a single species of plant. Rather, they will infect a group of closely related plant species. For example, the **tobacco mosaic virus** can infect plants of the genus *Nicotiana*. As the tobacco plant is one of the plants that can be infected, the virus has taken its name from that host. This name likely reflects the economic importance of the virus to the tobacco industry. Two other related viruses that were named for similar economic reasons are the potato-X and potato-Y viruses. The economic losses caused by these latter two viruses can be considerable. Some estimates have put the total worldwide damage as high as $60 billion a year.

The tobacco mosaic virus is also noteworthy as it was the first virus that was obtained in a pure form and in large quantity. This work was done by Wendall Meredith Stanley in 1935. For this and other work he received the 1946 Nobel Prize in Chemistry.

Plants infected with a virus can display lighter areas on leaves, which is called chlorosis. Chlorosis is caused by the degradation of the **chlorophyll** in the leaf. This reduces the degree of **photosynthesis** the plant can accomplish, which can have an adverse effect on the health of the entire plant. Infected plants may also display withered leaves, which is known as necrosis.

Sometimes plant viruses do not produce symptoms of infection. This occurs when the virus become latent. The viral nucleic acid becomes incorporated into the host material, just as happens with latent viruses that infect humans such as **herpes** viruses and **retroviruses**.

Most of the known plant viruses contain **ribonucleic acid** (**RNA**). In a virus known as the wound tumor virus, the RNA is present as a double strand. The majority of the RNA plant viruses, however, possess a single strand of the nucleic acid. A group of viruses known as gemini viruses contain single stranded **deoxyribonucleic acid** (**DNA**) as their genetic material, and the cauliflower mosaic virus contains double stranded DNA.

As with viruses of other hosts, plant viruses display different shapes. Also as with other viruses, the shape of any particular virus is characteristic of that species. For example, a tobacco mosaic virus is rod-shaped and does not display variation in this shape. Other plant viruses are icosahedral in shape (an icosahedron is a 20-sided figure constructed of 20 faces, each of which is an equilateral triangle).

There are no plant viruses known that recognize specific receptors on the plant. Rather, plant viruses tend to enter plant cells either through a surface injury to a leaf or the woody stem or branch structures, or during the feeding of an insect or the microscopic worms known as nematodes. These methods of transmission allow the virus to overcome the barrier imposed by the plant cell wall and cuticle layer. Those viruses that are transmitted by insects or animals must be capable of multiplication in the hosts as well as in the plant.

Plant viruses may also be transmitted to a new plant host via infected seeds from another plant. In the laboratory, viral DNA can be introduced into the bacterium *Agrobacterium tumefaciens*. When the bacterium infects a plant, the viral DNA can be incorporated into the plant genome. Experimental infection of plants can be done by rubbing virus preparation into the leaves of the plant. The virus can enter the plant through the physical abrasion that is introduced.

As humans can mount an immune response against viral infection, so plants have defense strategies. One strategy is the presence of a tough cell wall on many plants that restricts the entry of viruses unless the surface barrier of the plant is compromised, as by injury. Many plants also display a response that is termed hypersensitivity. In this response the plant cells in the vicinity of the infected cell die. This acts to limit the spread of the virus, since the virus require living cells in which to replicate.

Some plants have been shown to be capable of warning each other of the presence of a viral infection. This communication is achieved by the airborne release of a specific compound. This behavior is similar to the cell to cell signaling found in bacterial populations, which is known as **quorum sensing**.

See also Viral genetics; Virology

PLAQUE

Plaque is the diverse community of **microorganisms**, mainly **bacteria**, which develops naturally on the surface of teeth. The microbes are cocooned in a network of sugary polymers produced by the bacteria, and by host products, such as saliva, epithelial and other host cells, and inorganic compounds such as calcium. The surface-adherent, enmeshed community of plaque represents a biofilm.

Plaque is important for two reasons, one beneficial and the other detrimental. The beneficial aspect of dental plaque is that the coverage of the tooth surface by microbes that are normally resident in the host can exclude the colonization of the tooth by extraneous bacteria that might be harmful. This phenomenon is known as competitive exclusion. However, despite this benefit, the plaque can position acid-producing bacteria near the tooth and protect those bacteria from attempts to kill or remove them. Plaque can become extremely hard, as the constituent inorganic components create a crystalline barrier. Protected inside the plaque, the acid-producing bacteria can dissolve the tooth enamel, which can lead to the production of a cavity.

A plaque is a complex community, consisting of hundreds of species of bacteria. Plaque formation generally begins with the adherence of certain bacteria, such as *Streptococcus sanguis*, *Streptococcus mutans*, and *Actinomyces viscosus*. Then, so-called secondary colonizers become established. Examples include *Fusobacterium nucleatum* and *Prevotella intermedia*. As the plaque matures, a varied variety of other bacteria can colonize the tooth surface.

Maturation of the plaque is associated with a shift in the type of bacteria that are predominant. Gram-positive bacteria that can exist in the presence or absence of oxygen give way to gram negative bacteria that require the absence of oxygen.

Depending on how the community evolves, the plaque can become problematic in terms of a cavity. Even within the plaque, there are variations in the structure and bacterial composition. Thus, even though one region of the plaque is relatively benign is no guarantee that another region will house detrimental bacteria.

The prevalence of acid-producing bacteria is related to the diet. A diet that is elevated in the types of sugar typically found in colas and candy bars will lower the **pH** in the plaque. The lowered pH is harsh on all organisms except the acid-producing bacteria. Most dentists assert that a diet that contains less of these sugars, combined with good oral **hygiene**, will greatly minimize the threat posed by plaque and will emphasize the benefit of the plaque's presence.

See also Bacteria and bacterial infection; Biofilm formation and dynamic behavior; Microbial flora of the oral cavity, dental caries

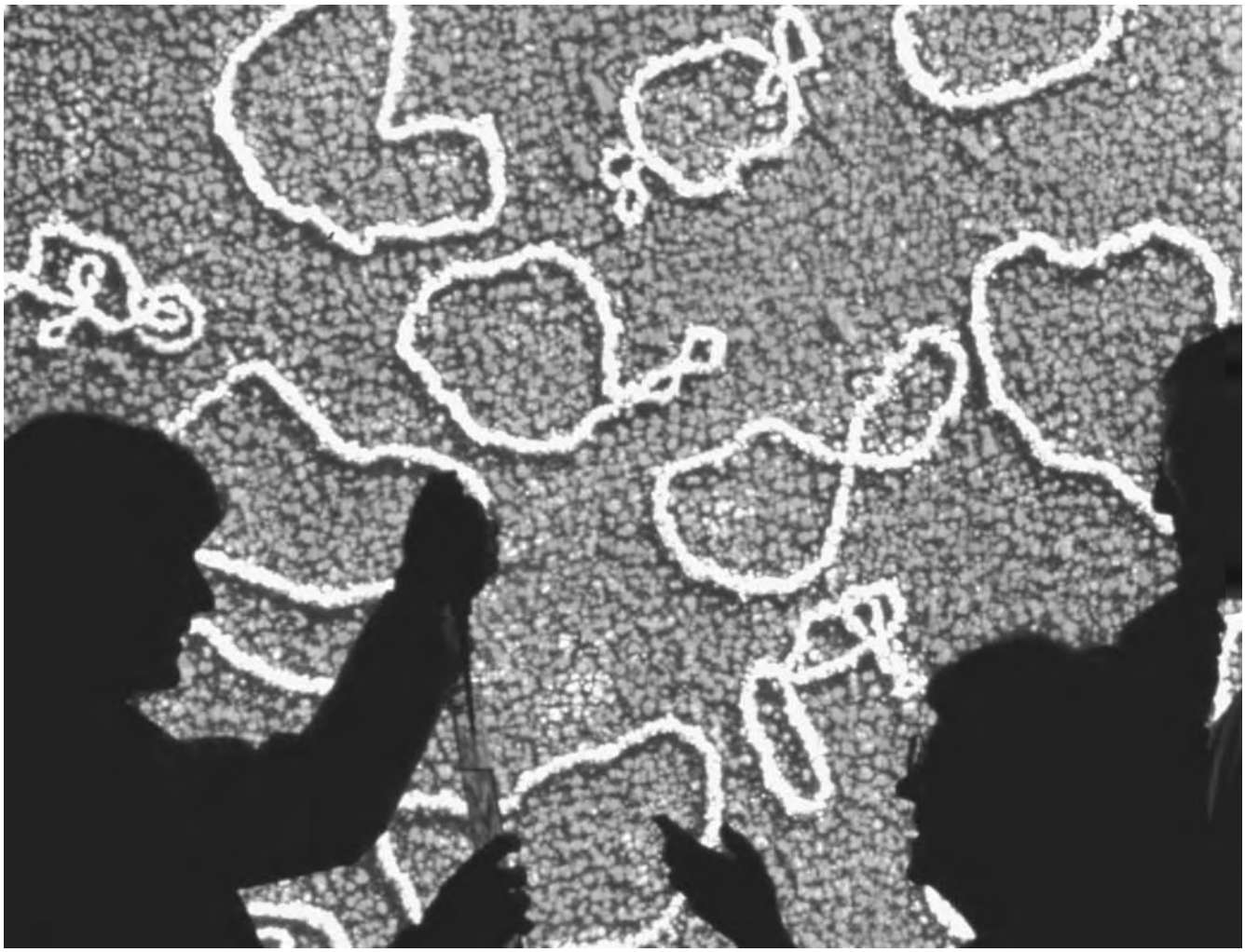
PLASMIDS

Plasmids are extra-chromosomal, covalently closed circular (CCC) molecules of double stranded (ds) **DNA** that are capable of autonomous replication. The prophages of certain bacterial phages and some dsRNA elements in **yeast** are also called plasmids, but most commonly plasmids refer to the extra-chromosomal CCC DNA in **bacteria**.

Plasmids are essential tools of genetic engineering. They are used as vectors in **molecular biology** studies.

Plasmids are widely distributed in nature. They are dispensable to their host cell. They may contain genes for a variety of phenotypic traits, such as **antibiotic resistance**, virulence, or metabolic activities. The products plasmids encode may be useful in particular conditions of **bacterial growth**. Replication of plasmid DNA is carried out by subsets of **enzymes** used to duplicate the bacterial chromosome and is under the control of plasmid's own replicon. Some plasmids reach copy numbers as high as 700 per cell, whereas others are maintained at the minimal level of 1 plasmid per cell. One particular type of plasmid has the ability to transfer copies of itself to other bacterial stains or species. These plasmids have a tra **operon**. Tra operon encodes the protein that is the component of sex pili on the surface of the host bacteria. Once the sex pili contact with the recipient cells, one strand of the plasmid is transferred to the recipient cells. This plasmid can integrate into the host chromosomal DNA and transfer part of the host DNA to the recipient cells during the next DNA transfer process.

Ideally, plasmids as vectors should have three characteristics. First, they should have a multiple **cloning** site (MSC) which consists of multiple unique restriction enzyme sites and allows the insertion of foreign DNA. Second, they should have a relaxed replication control that allows suffi-



Transmission electron micrograph of plasmids.

cient plasmids to be produced. Last, plasmids should have selectable markers, such as **antibiotics** metabolite genes, which allow the identification of the transformed bacteria. Numerous plasmid vectors have been developed since the first plasmid vectors of the early 1970s. Some vectors have **bacteriophage** promoter sequences flanking the MSC that allows direct sequencing of the cloned DNA sequence. Some vectors have yeast or **virus replication** origin, which allows the plasmids to replicate in yeast and mammalian cells, hence enabling cloned cDNAs to express in these host cells. Many new features have and will be added into plasmids to make genetic engineering easier and faster.

See also Cloning, application of cloning to biological problems; DNA (Deoxyribonucleic acid); DNA hybridization; Molecular biology and molecular genetics

PLASMODIUM

Plasmodium is a genus of **protozoa** that has a life cycle that includes a human host and a mosquito. The genus consists predominantly of four species: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. With the exception of the latter species, *Plasmodium* are **parasites** of humans.

The main disease of concern with *Plasmodium* is **malaria**. This disease has been a problem for humans for millennia. There are still almost 20 million cases of malaria reported each year. The number of people who are actually infected is thought to be upwards of 500 million people annually. The death toll from malaria is one to two million people each year, mostly in underdeveloped countries. But even in developed countries, malaria can be a problem, especially if mosquito control programs are not vigilant.

The protozoan is spread to humans by the bite of a female Anopheline mosquito. A form of the parasite known as

the sporozoite enters the bloodstream and makes its way to the liver. After multiplying in liver cells, the protozoan can penetrate red blood cells, which is a hallmark of the disease malaria. Multiplication occurs in a red blood cell, which ultimately bursts, releasing new forms of the protozoa that can infect neighbouring red blood cells. Such cycles lead to massive destruction of red blood cells.

Malaria can produce a myriad of symptoms, including high fever, generalized aches, tender spleen and liver, jaundice, and, in severe cases, convulsions, failure of the kidneys, shock, and collapse of the circulatory system. The fever tends to be cyclical, reflecting the cyclical pattern of protozoan release from red blood cells followed by a period of protozoan multiplication inside other red blood cells. These cycles can vary from 48 hours with *Plasmodium vivax* to about 72 hours with *Plasmodium malariae*.

Resistance of the protozoa, particularly *Plasmodium falciparum*, to the drugs such as chloroquinine and pyrimethamine that have previously been an effective control was first reported in 1961. Since that time, the occurrence of resistance has increased. A major factor in the development of the resistance is the adaptivity of the protozoan. The genome of the *Plasmodium* is very complex, and genetic alteration to new environmental pressures occurs quickly.

See also Parasites; Zoonoses

PLASTID • *see* PLASMIDS

PNEUMONIA, BACTERIAL AND VIRAL

Pneumonia is an infection of the lung, and can be caused by nearly any class of organism known to cause human infections, including **bacteria**, **viruses**, **fungi**, and **parasites**. In the United States, pneumonia is the sixth most common disease leading to death, and the most common fatal infection acquired by already hospitalized patients. In developing countries, pneumonia ties with diarrhea as the most common cause of death.

The main function of the respiratory system is to provide oxygen, the most important energy source for the body's cells. Inspired air travels down the respiratory tree to the alveoli, where the oxygen moves out of the alveoli and is sent into circulation throughout the body as part of the red blood cells. The oxygen in the inspired air is exchanged within the alveoli for the body's waste product, carbon dioxide, which leaves the alveoli during expiration.

The normal, healthy human lung is sterile, meaning that there are no normally resident bacteria or viruses (unlike the upper respiratory system and parts of the gastrointestinal system, where bacteria dwell even in a healthy state). There are multiple safeguards along the path of the respiratory system that are designed to keep invading organisms from leading to infection.

The first line of defense includes the hair in the nostrils, which serves as a filter for larger particles. The epiglottis is a trap door of sorts, designed to prevent food and other swallowed substances from entering the larynx and then trachea. Sneezing and coughing, both provoked by the presence of irritants within the respiratory system, help to clear such irritants from the respiratory tract.

Mucous, produced throughout the respiratory system, also serves to trap dust and infectious organisms. Tiny hair-like projections (cilia) from cells lining the respiratory tract beat constantly, moving debris, trapped by mucus, upwards and out of the respiratory tract. This mechanism of protection is referred to as the mucociliary escalator.

Cells lining the respiratory tract produce several types of immune substances which protect against various organisms. Other cells (called macrophages) along the respiratory tract actually ingest and kill invading organisms.

The organisms that cause pneumonia, then, are usually carefully kept from entering the lungs by virtue of these host defenses. However, when an individual encounters a large number of organisms at once, either by inhaling contaminated air droplets, or by aspiration of organisms inhabiting the upper airways, the usual defenses may be overwhelmed and infection may occur.

In addition to exposure to sufficient quantities of causative organisms, certain conditions may predispose an individual to pneumonia. Certainly, the lack of normal anatomical structure could result in an increased risk of pneumonia. For example, there are certain inherited defects of cilia which result in less effective protection. Cigarette smoke, inhaled directly by a smoker or second-hand by an innocent bystander, interferes significantly with ciliary function, as well as inhibiting macrophage function.

Stroke, seizures, alcohol, and various drugs interfere with the function of the epiglottis, leading to a leaky seal on the trap door, with possible **contamination** by swallowed substances and/or regurgitated stomach contents. Alcohol and drugs also interfere with the normal cough reflex, further decreasing the chance of clearing unwanted debris from the respiratory tract.

Viruses may interfere with ciliary function, allowing themselves or other microorganism invaders, such as bacteria, access to the lower respiratory tract. One of the most important viruses which in recent years has resulted in a huge increase in the incidence of pneumonia is **HIV** (**Human Immunodeficiency Virus**), the causative virus in **AIDS** (Acquired Immunodeficiency Syndrome). Because AIDS results in a general decreased effectiveness of many aspects of the host's **immune system**, a patient with AIDS is susceptible to all types of pneumonia, including some previously rare parasitic types which would be unable to cause illness in an individual possessing a normal immune system.

The elderly have a less effective mucociliary escalator, as well as changes in their immune system, all of which cause them to be more at risk for the development of pneumonia.

Various chronic conditions predispose to pneumonia, including asthma, cystic fibrosis, neuromuscular diseases which may interfere with the seal of the epiglottis, and esophageal disorders which result in stomach contents passing upwards into the esophagus (increasing the risk of aspiration of those stom-

ach contents with their resident bacteria), as well as diabetes, sickle cell anemia, lymphoma, leukemia, and emphysema.

Pneumonia is one of the most frequent infectious complications of all types of surgeries. Many drugs used during and after surgery may increase the risk of aspiration, impair the cough reflex, and cause a patient to underfill their lungs with air. Pain after surgery also discourages a patient from breathing deeply and coughing effectively.

The list of organisms which can cause pneumonia is very large, and includes nearly every class of infecting organism: viruses, bacteria, bacteria-like organisms, fungi, and parasites (including certain worms). Different organisms are more frequently encountered by different age groups. Furthermore, other characteristics of the host may place an individual at greater risk for infection by particular types of organisms.

Viruses, especially respiratory syncytial virus, parainfluenza and **influenza** viruses, and adenovirus, cause the majority of pneumonias in young children. Pneumonia in older children and young adults is often caused by the bacteria-like *Mycoplasma pneumoniae*. Adults are more frequently infected with bacteria (such as *Streptococcus* pneumoniae, *Hemophilus inflenzae,* and *Staphylococcus aureus*).

The parasite *Pneumocystis carinii* is an extremely important cause of pneumonia in patients with immune problems, such as patients being treated for cancer with **chemotherapy**, or patients with AIDS. People who have reason to come in contact with bird droppings, such as poultry workers, are at risk for pneumonia caused by the parasite *Chlamydia psittaci*. A very large, serious outbreak of pneumonia occurred in 1976, when many people attending an American Legion convention were infected by a previously unknown organism (subsequently named *Legionella pneumophila*) which was traced to air conditioning units in the convention hotel.

Pneumonia is suspected in any patient who presents with fever, cough, chest pain, shortness of breath, and increased respirations (number of breaths per minute). Fever with a shaking chill is even more suspicious, and many patients cough up clumps of mucus (sputum) that may appear streaked with pus or blood. Severe pneumonia results in the signs of oxygen deprivation, including blue appearance of the nail beds (cyanosis).

The invading organism causes symptoms, in part, by provoking an overly exuberant immune response in the lungs. The small blood vessels in the lungs (capillaries) become leaky, and protein-rich fluid seeps into the alveoli. This results in less functional area for oxygen-carbon dioxide exchange. The patient becomes relatively oxygen deprived, while retaining potentially damaging carbon dioxide. The patient breathes faster, in an effort to bring in more oxygen and blow off more carbon dioxide.

Mucus production is increased, and the leaky capillaries may tinge the mucus with blood. Mucus plugs actually further decrease the efficiency of gas exchange in the lung. The alveoli fill further with fluid and debris from the large number of white blood cells being produced to fight the infection.

Consolidation, a feature of bacterial pneumonias, occurs when the alveoli, which are normally hollow air spaces within the lung, instead become solid, due to quantities of fluid and debris.

Viral pneumonias and mycoplasma pneumonias do not result in consolidation. These types of pneumonia primarily infect the walls of the alveoli and the parenchyma of the lung.

Diagnosis is for the most part based on the patient's report of symptoms, combined with examination of the chest. Listening with a stethoscope will reveal abnormal sounds, and tapping on the patient's back (which should yield a resonant sound due to air filling the alveoli) may instead yield a dull thump if the alveoli are filled with fluid and debris.

Laboratory diagnosis can be made of some bacterial pneumonias by staining sputum with special chemicals and looking at it under a **microscope**. Identification of the specific type of bacteria may require culturing the sputum (using the sputum sample to grow greater numbers of the bacteria in a lab dish).

X-ray examination of the chest may reveal certain abnormal changes associated with pneumonia. Localized shadows obscuring areas of the lung may indicate a bacterial pneumonia, while streaky or patchy appearing changes in the x-ray picture may indicate viral or mycoplasma pneumonia. These changes on x-ray, however, are known to lag in time behind the patient's actual symptoms.

Antibiotics, especially given early in the course of the disease, are very effective against bacterial causes of pneumonia. Erythromycin and tetracycline improve recovery time for symptoms of mycoplasma pneumonia, but do not eradicate the organisms. Amantadine and acyclovir may be helpful against certain viral pneumonias.

Because many bacterial pneumonias occur in patients who are first infected with the influenza virus (the flu), yearly **vaccination** against influenza can decrease the risk of pneumonia for certain patients, particularly the elderly and people with chronic diseases (such as asthma, cystic fibrosis, other lung or heart diseases, sickle cell disease, diabetes, kidney disease, and forms of cancer). A specific **vaccine** against *Streptococcus* pneumoniae is very protective, and should be administered to patients with chronic illnesses. Patients who have decreased immune resistance (due to treatment with chemotherapy for various forms of cancer or due to infection with the AIDS virus), and therefore may be at risk for infection with *Pneumocystis carinii,* are frequently put on a regular drug regimen of Trimethoprim sulfa and/or inhaled pentamidine to avoid Pneumocystis pneumonia.

POLIOMYELITIS AND POLIO

Poliomyelitis is a contagious infectious disease that is caused by three types of poliovirus. The **viruses** cause damage and destruction of cells in the nervous system. Paralysis can result in about 2% of those who contract the disease, which is called polio. Most people who contract polio either have mild symptoms or no symptoms at all.

Poliomyelitis has been part of human history for millennia. An Egyptian stone engraving depicting the debilitating effects of poliomyelitis dates from over 3,000 years ago. In that time, the occurrence of polio was rare, as sanitation was poor. The close proximity between people and raw sewage bestowed protective **immunity** against the polioviruses, which reside in the feces. As sewage treatment became better and indoor plumbing became widespread in the twentieth century, exposure to the virus became less and the protective immunity was less likely to develop in children. By the time the disease was first described in Britain in 1789 by Michael Underwood, outbreaks in children were occurring. From the latter decades of the nineteenth century through to the 1950s, polio **epidemics** occurred frequently. Children were most at risk and could be crippled from polio, or suffer muscle damage severe enough to require the assistance of iron lungs, early mechanical ventilators, because their lungs had been damaged to the point of incapacity.

The word poliomyelitis derives from the Greek word *polio* (grey) and *myelon* (marrow, indicating the spinal cord). It is the poliomyelitis effect on the spinal cord that is associated with the devastating paralysis of the severe form of the disease.

Poliovirus is a member of the enterovirus group of the family Picornaviridae. Poliovirus serotypes (an antigenic means of categorizing viruses) P1, P2, and P3 are the agents of poliomyelitis. The viruses are very susceptible to heat, ultraviolet light and chemicals such as formaldehyde and chlorine.

Poliovirus is spread most commonly via contact with feces. Only humans are involved in the transmission, as only humans harbor the virus. Typically, feces-soiled hands are not washed properly and then are put into or round the mouth. Spread of the virus by coughing or sneezing can also occur. The virus multiplies in the pharynx or the gastrointestinal tract. From there the virus invades adjacent lymph tissue, enters the blood stream and can infect cells of the central nervous system. When neurons of the brain stem are infected, the paralytic symptoms of poliomyelitis result.

About 95% of those who are infected by the poliovirus may not exhibit symptoms. In those people who do exhibit symptoms, about 4–8% exhibit a fever and flu-like malaise and nausea. Recovery is complete within a short time. These people can continue to excrete the virus in their feces for a time after recovery, and so can infect others. About 2% of those with symptoms develop a more sever form of nonparalytic aseptic **meningitis**. The symptoms include muscular pain and stiffness. In the severe paralytic poliomyelitis, which occurs in less than 2% of all polio infections, breathing and swallowing become difficult and paralysis of the bladder and muscles occurs. Paralysis of the legs and the lung muscles is common. This condition is known as flaccid paralysis.

The paralytic form of polio can be of three types. Spinal polio is the most common, accounting for 79% of paralytic polio in the Unites States from 1969 to 1979. Bulbar polio, which accounts for 2% of cases, produces weakness in those muscles that receive impulses from the cranial nerves. Finally, bulbospinal polio, which is a combination of the two, accounts for about 20% of all cases.

At the time of the development of the vaccines to polio, in the early 1950s, there were nearly 58,000 cases of polio annually in the United States, with almost 20,000 of these people being rendered paralyzed. Earlier, President Franklin Roosevelt committed funds to a "war on polio." Roosevelt was himself a victim of polio.

Jonas Salk developed a **vaccine** to the three infectious forms of the poliovirus (out of the 125 known strains of the virus) in the early 1950s. His vaccine used virus that had been inactivated by the chemical formaldehyde. An immune response was still mounted to the virus particles when they were injected into humans. The vaccine was effective (except for one early faulty batch) and quickly became popular.

Soon after the Salk vaccine appeared, **Albert Sabin** developed a vaccine that was based on the use of still-live, but weakened, polio virus. The vaccine was administered as an oral solution. While effective as a vaccine, the weakened virus can sometimes mutate to a disease-causing form, and the vaccine itself, rarely, can cause poliomyelitis (vaccine-associated paralytic poliomyelitis). As of January 2000, the **Centers for Disease Control** has recommended that only the Salk version of the polio vaccine be used.

There is still no cure for poliomyelitis. In the post-vaccine era, however, poliomyelitis is virtually non-existent in developed countries. For example, in the United States there are now only approximately eight reported cases of polio each year, mostly due to the vaccine-associated paralytic phenomenon. The last cases of poliomyelitis in the Unites States caused by wild virus occurred in 1979. Elsewhere, there are still bout 250,00 cases every year, mainly in the India subcontinent, the Eastern Mediterranean, and Africa. However, an ongoing **vaccination** campaign by the **World Health Organization** aims to eradicate poliomyelitis by 2010.

See also History of public health

Polymerase Chain Reaction (PCR)

PCR (polymerase chain reaction) is a technique in which cycles of denaturation, annealing with primer, and extension with **DNA** polymerase, are used to amplify the number of copies of a target DNA sequence by more than 106 times in a few hours. American molecular biologist Kary Mullis developed the idea of PCR in the 1970s. For his ingenious invention, he was awarded the 1993 Nobel Prize in physiology or medicine.

The extraction of DNA polymerase from thermophilic **bacteria** allowed major advances in PCR technology.

PCR amplification of DNA is like any DNA replication by DNA polymerase *in vivo* (in living cells). The difference is that PCR produces DNA in a test tube. For a PCR to happen, four components are necessary: template, primer, deoxyribonecleotides (adenine, thymine, cytosine, guanine), and DNA polymerase. In addition, part of the sequence of the targeted DNA has to be known in order to design the according primers. In the first step, the targeted double stranded DNA is heated to over 194°F (90°C) for denaturation. During this

process, two strands of the targeted DNA are separated from each other. Each strand is capable of being a template. The second step is carried out around 122°F (50°C). At this lowered temperature, the two primers anneal to their complementary sequence on each template. The DNA polymerase then extends the primer using the provided nucleotides. As a result, at the end of each cycle, the numbers of DNA molecules double.

PCR was carried out manually in incubators of different temperatures for each step until the discovery of DNA polymerase from thermophilic bacteria. The bacterium *Thermus aquaticus* was found in Yellow Stone National Park. This bacterium lives in the hot springs at 203°F (95°C). The DNA polymerase from *T. aquaticus* keeps its activity at above 203°F (95°C) for many hours. Several additional heat-resistant DNA polymerases have also now been identified.

Genetic engineered heat resistant DNA polymerases, that have proofreading functions and make fewer **mutations** in the amplified DNA products, are available commercially. PCR reactions are now carried out in different thermocyclers. Thermocyclers are designed to change temperatures automatically. Researchers set the temperatures and the time, and at the end of the procedure take the test tube out of the machine.

The invention of PCR was revolutionary to **molecular biology**. PCR is valuable to researchers because it allows them to multiply the quantity of a unique DNA sequence to a large and workable amount in a very short time. Researchers in the Human Genome Project are using PCR to look for markers in cloned DNA segments and to order DNA fragments in libraries. Molecular biologists use PCR to **cloning** DNA. PCR is also used to produce biotin or other chemical-labeled probes. These probes are used in nucleic acid hybridization, *in situ* hybridization and other molecular biology procedures.

PCR, coupled with fluorescence techniques and computer technology, allows the real time amplification of DNA. This enables quantitative detection of DNA molecules that exist in minute amounts. PCR is also used widely in clinical tests. Today, it has become routine to use PCR in the diagnosis of infectious diseases such **AIDS**.

See also Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); DNA chips and micro arrays; DNA hybridization; Immunogenetics; Laboratory techniques in immunology; Laboratory techniques in microbiology; Molecular biology and molecular genetics

PORINS

Porins are proteins that are located in the outer membrane of Gram-negative **bacteria**. They function to form a water-filled pore through the membrane, from the exterior to the **periplasm**, which is a region located between the outer and inner membranes. The porin channel allows the diffusion of small hydrophilic (water-loving) molecules through to the periplasm. The size of the diffusing molecule depends on the size of the channel.

A porin protein associates with two other porin proteins of the same type in the outer membrane. This may act to stabilize the three-dimensional structure of each porin molecule. Each porin contains a pore, so that there are three pores in the triad of porins.

The size of the water-filled channel that is created by a porin depends on the particular porin protein. For example, in the bacterium ***Escherichia coli***, the so-called maltoporin and phosphoporin have different specificities (for the sugar maltose and phosphorus, respectively).

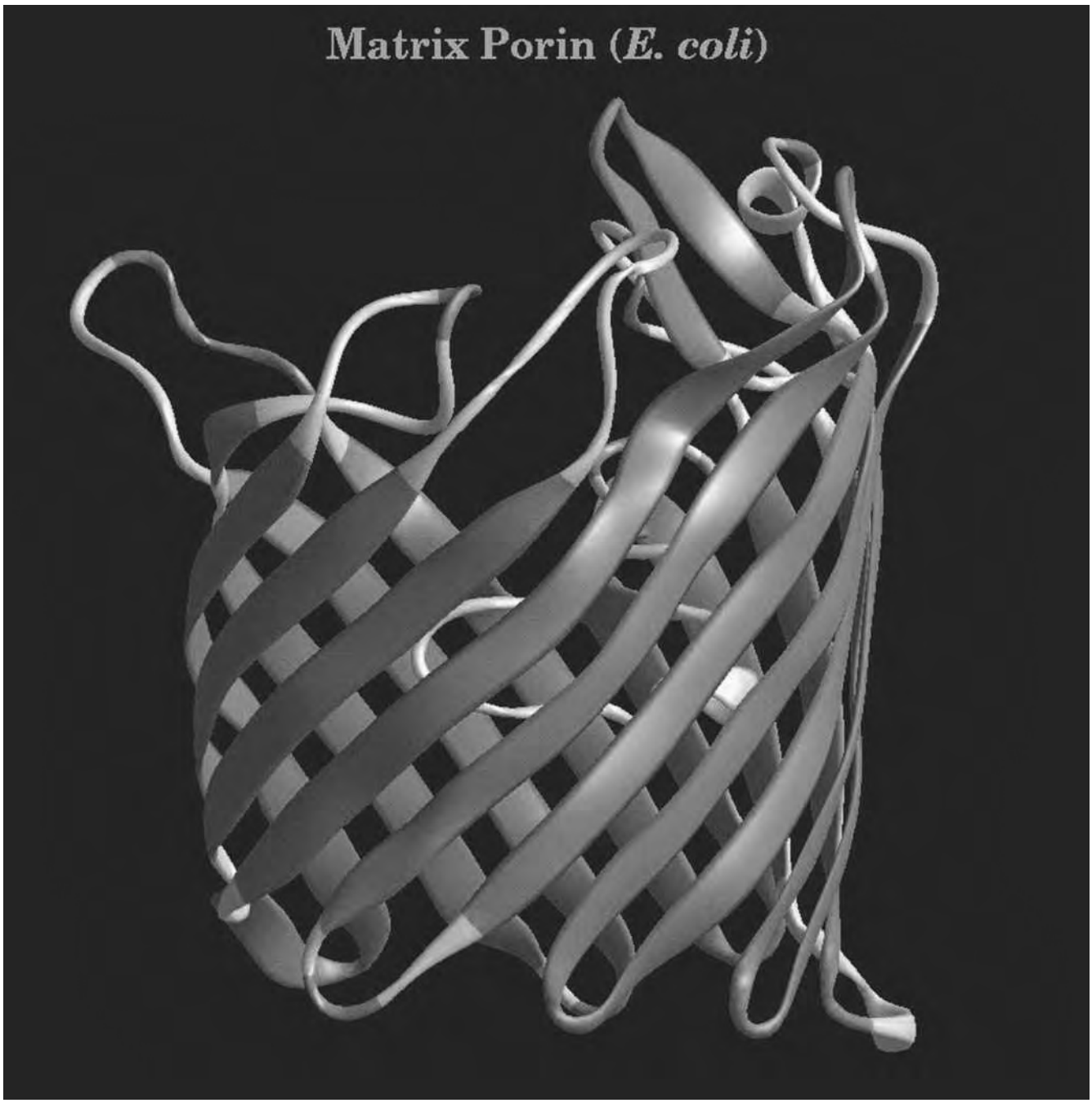
Since the discovery of porins in the 1970s in *Escherichia coli*, these proteins have been shown to be a general feature of the Gram-negative outer membrane. Much of the early work on porins came from the laboratories of Hiroshi Nikaido and Robert Hancock. Some examples of the bacteria known to possess porins are *Pseudomonas aeruginosa*, many other species of *Pseudomonas*, *Aeromonas salmonicida*, *Treponema pallidum*, and *Helicobacter pylori*.

A bacterium typically contains a variety of porins. Possession of porins of different sizes and chemistries is very advantageous for a bacterium. The various channels allow for the inward diffusion of a variety of nutrients required by the bacterium for survival and growth. Moreover, the diffusional nature of the molecule's entry means that a bacterium is able to acquire some needed nutrients without having to expend energy.

Another example of porin importance is found in *Escherichia coli*. In this bacterium, a duo of porins, which are designated OmpF and OmpC, function in response to changes in osmolarity. The production of these porins is under the control of a protein that senses the osmotic character of the environment. Depending on the ionic conditions, the amounts of OmpF and OmpC in the outer membrane can be altered so as to control the types of ions that enter the bacterium.

Porins share the same function in these bacteria from various habitats. This similar function is mirrored by the similarity in the three-dimensional structure of the proteins. Each porin is visually reminiscent of a barrel that is open to both ends. The slats of the barrel are arrangements of the constituent amino acids of the protein (beta sheets). The sequence of amino acids that makes up a beta sheet region allows the region to assume a zigzag configuration of the amino acids in one plane. The result is a narrow, flat strip of amino acids. When such strips are linked together, the barrel shape can be created. The outer surface of the porin barrel is more **hydrophobic** (water-hating) and so the partitioning of this surface into the hydrophobic interior of the membrane will be favored. The inner surface of the porin barrel contains side groups of the amino acids that prefer to interact with water.

The function of porin proteins was discovered by isolating the particular protein and then inserting the protein into model systems, that consisted either of lipid membranes floating in solution (liposomes) or floating as a sheet on the surface of a liquid (black lipid bilayer membranes). The passage of radioactive sugars of various sizes out of the liposomes or across the black lipid bilayer membranes could be measured, and the various so-called exclusion limits for each porin could be determined.



Three-dimensional computer model of a protein molecule of matrix porin found in *E. coli* bacteria.

Porins also have relevance in the **antibiotic resistance** of bacteria, particularly *Pseudomonas aerugionsa*, which is the cause of lung infections in those afflicted with cystic fibrosis, and can cause so-called "opportunistic infections" in those whose **immune system** is impaired. For example, the porin designated OprD is specifically utilized for the diffusion of the antibiotic imipenem into the bacterium. Imipenem resistance is associated with an alteration in the three-dimensional structure of OprD such that imipenem is excluded from entering the bacterium. The resistance of a number of clinical isolates of *Pseudomonas aeruginosa* is the result of porin alteration. Knowledge of the molecular nature of the alterations will help in the design of **antibiotics** that overcome the channel barrier.

See also Bacterial membranes and cell wall; Protein export

PRESUMPTIVE TESTS • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

PRIONS

Prions are proteins that are infectious. Indeed, the name prion is derived from "proteinaceous infectious particles." The discovery of prions and confirmation of their infectious nature overturned a central dogma that infections were caused by intact organisms, particularly **microorganisms** such as **bacteria**, **fungi**, **parasites**, or **viruses**. Since prions lack genetic material, the prevailing attitude was that a protein could not cause disease.

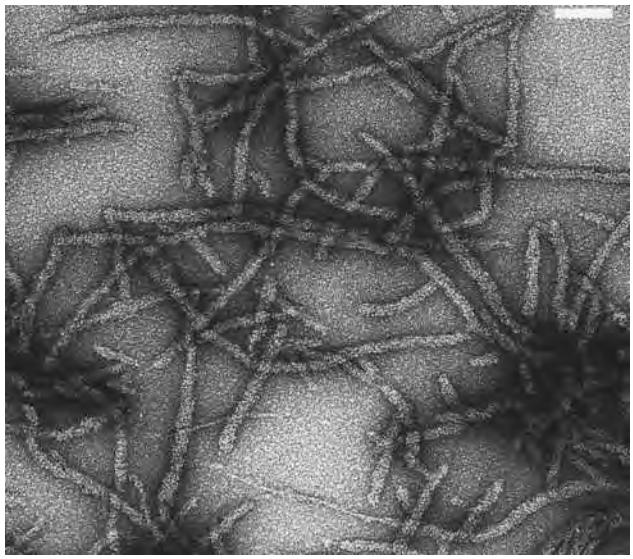
Prions were discovered and their role in brain degeneration was proposed by Stanley Pruisner. This work earned him the 1997 Nobel Prize in medicine or physiology.

In contrast to infectious agents that are not normal residents of a host, prion proteins are a normal constituent of brain tissue in humans and in all mammals studied thus far. The prion normally is a constituent of the membrane that surrounds the cells. The protein is also designated PrP (for proteinaceous infectious particle). PrP is a small protein, being only some 250 amino acids in length. The protein is arranged with regions that have a helical conformation and other regions that adopt a flatter, zigzag arrangement of the amino acids. The normal function of the prion is still not clear. Studies from mutant mice that are deficient in prion manufacture indicate that the protein may help protect the brain tissue from destruction that occurs with increasing frequency as someone ages. The normal prions may aid in the survival of brain cells known as Purkinje cells, which predominate in the cerebellum, a region of the brain responsible for movement and coordination.

The so-called prion theory states that PrP is the only cause of the prion-related diseases, and that these disease results when a normally stable PrP is "flipped" into a different shape that causes disease. Regions that are helical and zigzag are still present, but their locations in the protein are altered. This confers a different three-dimensional shape to the protein.

As of 2002, the mechanism by which normally functioning protein is first triggered to become infectious is not known. One hypothesis, known as the virino hypothesis, proposes that the infectious form of a prion is formed when the PrP associates with nucleic acid from some infectious organism. Efforts to find prions associated with nucleic acid have, as of 2001, been unsuccessful.

If the origin of the infectious prion is unclear, the nature of the infectious process following the creation of an infectious form of PrP is becoming clearer. The altered protein is able to stimulate a similar structural change in surrounding prions. The change in shape may result from the direct contact and binding of the altered and infectious prion with the unaltered and still-normally functioning prions. The altered proteins also become infective and encourage other proteins to undergo the conformational change. The cascade produces proteins that adversely effect neural cells and the cells lose their ability to function and die.



Negative stain electron micrograph of prions.

The death of regions of the brain cells produces holes in the tissue. This appearance leads to the designation of the disease as spongiform encephalopathy.

The weight of evidence now supports the contention that prion diseases of animals, such as scrapie in sheep and bovine spongiform encephalopathy (BSE—popularly known as Mad cow disease) can cross the species barrier to humans. In humans, the progressive loss of brain function is clinically apparent as Creutzfeld-Jacob disease, kuru, and Gerstmann-Sträussler-Scheinker disease. Other human disease that are candidates (but as yet not definitively proven) for a prion origin are Alzheimer's disease and Parkinson's disease.

In the past several years, a phenomenon that bears much similarity to prion infection has been discovered in **yeast**. The prion-like protein is not involved in a neurological degeneration. Rather, the microorganism is able to transfer genetic information to the daughter cell by means of a shape-changing protein, rather than by the classical means of genetic transfer. The protein is able to stimulate the change of shape of other proteins in the interior of the daughter cell, which produces proteins having a new function.

The recent finding of a prion-related mechanism in yeast indicates that prions my be a ubiquitous feature of many organisms and that the protein may have other functions than promoting disease.

See also BSE and CJD disease; BSE and CJD disease, advances in research

PROBIOTICS

Probiotics is a term that refers to the consumption of certain **microorganisms** in an effort to improve overall health and the functioning of the body's microflora.

The use of microorganisms as a health aid is not new. People have asserted the health fortifying attributes of yogurt and fermented milk for thousands of years. However, the cause of the beneficial effect was unknown. A century ago, the Russian microbiologist **Élie Metchnikoff** (1845–1916) began the scientific assessment of the probiotic role of microorganisms.

Based on Metchnikoff's work and that of others, it appeared well established (but not clinically proven) by the 1920s that *Lactobacillus acidophilus* acted to relieve the conflicting conditions of constipation and diarrhea. Capsules containing living **bacteria** were popular items in drug stores of the day. However, with the advent of **antibiotics** as a cure for many ailments, the public interest in probiotics waned. The emphasis shifted to the treatment of infections, as opposed to the prevention of infections.

In the 1990s the interest in probiotics surged. A number of studies established the clinical significance of *Lactobacillus* and *Bifidobacterium* in improving the efficiency of lactose absorption, in the treatment of diarrhea in children, and in combating recurrent vaginal **yeast** infections.

Probiotic bacteria exert their effect by colonizing surfaces, such as found in the intestinal tract or the vagina. Compounds can also be produced by the adherent bacteria that are inhibitory to other types of bacteria. The net effect of these processes is the competitive exclusion of potentially harmful bacteria by the beneficial probiotic bacteria.

The exclusion process can extend to other infectious agents as well. For example, colonization of the intestinal tract with *Lactobacillus* GG has been shown to significantly reduce the length of diarrheal illness caused by rotavirus. The rotavirus had no place to adhere and were washed out of the intestinal tract.

Probiotics also have shown potential in relieving skin disorders that are the result of an allergic reaction to a food. The colonization of the intestinal wall appears to restore the ability of nutrients to cross from the intestinal canal to the bloodstream. This ability to absorb food nutrients is disrupted in those with some food **allergies**.

The molecular basis for the competitive exclusion behavior of bacteria such as *Lactobacillus* and *Bifidobacterium* is the subject of continuing study. The identification of the precise molecular agents that are responsible for surface blocking will expand the use of probiotics.

See also Lactobacillus; Microbial flora of the stomach and gastrointestinal tract

PROKARYOTAE

Prokaryote is a kingdom, or division, in the classification scheme devised for all life on Earth. This kingdom, which is also designated as Monera, includes all **bacteria** and **blue-green algae** (which are also called Cyanobacteria). There are four other kingdoms in the classification system. The classification is based on the structure of a subunit of the ribosome. This criterion was selected because the structure of the subunit seems to have been maintained with little change throughout the millions of years that life has existed on Earth.

Besides the kingdom Prokaryotae, there are the Protista (eukaryotic organisms' organisms that have a **nucleus** enclosed in a well-defined membrane), **Fungi**, Animalia (**eukaryotes** organized into complex organisms), and Plantae.

The use of kingdoms in the classification of organisms arose with the work of Carolus Linneus who, in the mid-1700s, devised the system that is still used today. The Linnean system of classification has kingdoms as the highest level, with six other subdivisions down to the species level. Bacteria are divided into various genera. A group of bacteria derived from a single cell is called a strain. Closely related strains constitute a bacterial species. For example, the complete classification of the bacterium ***Escherichia coli*** is as follows:

- Kingdom: Prokaryotae (Monera)
- Division (also called Phylum): Gracilicutes
- Class: Scotobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: *Escherichia*
- Species: *Escherichia coli*

The Prokaryotae are further divided into two subkingdoms. These are called the Eubacteriobonta (which contains the so-called **Eubacteria**) and the Archaebacteriobonta (which contains the so-called Archaebacteria). This split arose from the research of Carl Woese. He showed that the so-called 16 S ribosomal subunit of bacteria divide bacteria into two groups; the Eubacteria and the Archaeobcteria.

Archaebacteria are a very diverse group of bacteria and have several features that set them apart from the other Prokaryotae. Their cell walls lack a structure called the **peptidoglycan**, which is a rigid and stress-bearing network necessary for the survival of other bacteria. Archaebacteria live in extreme environments such as deep-sea vents, hot springs, and very salty water. Finally, some metabolic processes of Archaebacteria are different from other bacteria.

The feature that most distinguishes the bacteria and blue-green algal members of the Prokaryote from the members of the other kingdoms is the lack of membrane-bound structure around the genetic material. The genetic material, **deoxyribonucleic acid** (**DNA**), is dispersed through the inside of the microorganism, a region that is typically referred to as the **cytoplasm**. In contrast, eukaryotic organisms have their genetic material compartmentalized inside a specialized membrane.

A second distinctive feature of the Prokaryotae concerns their method of reproduction. Most bacteria reproduce by growing and then splitting in two. This is called binary fission. Eukaryotic organisms have a more complex process that involves the replication of their differently organized genetic material and the subsequent migration of the material to specific regions of the cell.

Blue-green algae and some bacteria are able to manufacture their own food from sunlight through the process of **photosynthesis**. Green plants likewise have this capability. This type of bacteria are the photoautotrophs. Other bacteria are able to utilize elements like nitrogen, sulphur, hydrogen, or iron to make their food. This type of Prokaryote are the chemoautotrophs. But the bulk of the Prokaryotae exists by decomposing and using compounds made by other organisms. This decomposition is a vital process. Without this bacterial activity, the wastes of other organisms would blanket Earth.

The relative simplicity of the Prokaryotae, as compared to eukaryotes, extends to the genetic level. The prototypical bacterial species *Escherichia coli* contains approximately 5,000 genes. On average, about one in every 200 bacteria is likely to have a mutation in at least one of the genes. In a 100 ml **culture** containing one million bacteria per milliliter, this translates to 500,000 mutant bacteria. This ability of members of the Prokaryotae to mutant and so quickly adapt to a changing environment is the principle reason for their success through time.

The ecological distribution of the Prokaryotae is vast. Bacteria have adapted to live almost everywhere, in environments as diverse as the thermal deep-sea vents to the boiling hot springs of Yellowstone National Park, from the soil to the intestinal tract of man and animals. The diversity of bacteria led to the design of a classification system just for them. **David Hendricks Bergey** spearheaded this classification scheme in the first half of the twentieth century. His efforts culminated in the publication (and ongoing revisions) of the *Bergey's Manual of Systematic Bacteriology*.

See also Bacterial kingdoms; Evolutionary origin of bacteria and viruses

PROKARYOTIC CELLS, GENETIC REGULATION OF

• *see* GENETIC REGULATION OF PROKARYOTIC CELLS

PROKARYOTIC MEMBRANE TRANSPORT

The ability of Prokaryotic **microorganisms** to move compounds into the cell, and to remove waste products of **metabolism** out of the cell, is crucial for the survival of the cell. Some of these functions are achieved by the presence of water-filled channels, particularly in the outer membrane of Gram-negative **bacteria**, which allow the diffusion of molecules through the channel. But this is a passive mechanism and does not involve the input of energy by the bacterium to accomplish the movement of the molecules across the membrane. Mechanisms that depend on the input of energy from the microorganism are active membrane transport mechanisms.

Prokaryotic membrane transport depends on the presence of specific proteins. These proteins are located within a membrane that surrounds the cell. Gram-positive bacteria have only a single membrane surrounding the contents of the bacterium. So, it is within this membrane that the transport proteins reside. In Gram-negative bacteria, the transport proteins are important constituents of the inner of the two membranes that are part of the cell wall. The inner membrane is also referred to as the cytoplasmic membrane.

There are a number of proteins that can participate in transport of molecules across Prokaryotic membranes. Different proteins have different modes of operation. In general, there are three different functional types of protein. These are termed uniporters, antiporters, and symporters.

Uniporters can actually be considered analogous to the water-filed channels of the Gram-negative outer membrane, in that a uniporter is a single protein or a collection of several like proteins that produces a channel through which molecules can passively diffuse. No energy is required for this process. Some degree of selectivity as to the types of molecules that can pass down a channel is achieved, based on the diameter of the channel. Thus, a small channel excludes large molecules.

A uniporter can also function in a process known as facilitated diffusion. This process is governed by the concentrations of the molecule of interest on either side of the membrane. If the concentration on one side of the membrane barrier is higher than on the other side, the movement of molecules through the connecting channel will naturally occur, in order to balance the concentrations on both sides of the membrane.

An antiporter is a membrane protein that can transport two molecules across the membrane in which it is embedded at the same time. This is possible as one molecule is transported in one direction while the other molecule is simultaneously transported in the opposite direction. Energy is required for this process, and functions to allow a change in the shape of the protein or to permit all or part of the protein to swivel upon binding of the molecules to be transported. One model has the molecules binding to the protein that is exposed at either surface of the membrane, and then, by an internal rotation of the transport protein, both molecules are carried to the other membrane surface. Then, each molecule is somehow released from the transport protein.

The third type of transport protein is termed a symport. This type of protein can simultaneously transport two molecules across a membrane in the same direction. The most widely held model for this process has the molecules binding to the transport protein that is exposed on the external surface of the membrane. In an energy-dependent process, these molecules are driven through a central region of the protein to emerge on the opposite side of the membrane. The protein molecule remains stationary.

The energy for prokaryotic membrane transport can come from the breakdown, or hydrolysis, of an energy-containing molecule called adenosine triphosphate (ATP). The hydrolysis of ATP provides energy to move molecules from a region of lower concentration to a region of higher concentration (i.e., transport is against a concentration gradient).

Alternatively, energy for transport in the antiport and symport systems can be provided by the molecules themselves. The fact that the molecules prefer to be associated

with the protein, rather than in solution, drives the transport process.

The outer membrane of Gram-negative bacteria does contain proteins that participate in the active transport of specific molecules to the periplasmic space, which separates the outer and inner membranes. Examples of such transport proteins include the FhuA of ***Escherichia coli*** and FepA of this and other bacteria. This type of active transport is important for disease processes, as iron can be crucial in the establishment of an infections, and because available iron is normally in very low concentration in the body.

See also Bacterial membranes and cell wall; Protein export

PROKARYOTIC REPLICATION • *see* CELL CYCLE (PROKARYOTIC), GENETIC REGULATION OF

PROMED • *see* EPIDEMIOLOGY, TRACKING DISEASES WITH TECHNOLOGY

PROPRIONIC ACID BACTERIA • *see* ACNE, MICROBIAL BASIS OF

PROSTHECATE AND NON-PROSTHECATE APPENDAGES • *see* BACTERIAL APPENDAGES

PROTEIN CRYSTALLOGRAPHY

Protein crystallography is a technique that utilizes x rays to deduce the three-dimensional structure of proteins. The proteins examined by this technique must first be crystallized.

When x rays are beamed at a crystal, the electrons associated with the atoms of the crystal are able to alter the path of the x rays. If the x rays encounter a film after passing through the crystal, a pattern can be produced following the development of the film. The pattern will consist of a limited series of dots or lines, because a crystal is composed of many repeats of the same molecule. Through a series of mathematical operations, the pattern of dots and lines on the film can be related to the structure of the molecule that makes up the crystal.

Crystallography is a powerful tool that has been used to obtain the structure of many molecules. Crystallography data was used, for example, in the determination of the structure of the double helix of **deoxyribonucleic acid** by American molecular biologist James Watson and British molecular biologist **Francis Crick** in the 1950s. **Bacteria** and virus are also amenable to x-ray crystallography study. For example, the structure of the toxin produced by *Vibrio cholerae* has been deduced by this technique. Knowledge of the shape of cholera toxin will help in the tailoring of molecules that will bind to the active site of the toxin. In this way, the toxin's activity can be neutralized. Another example is that of the tail region of the virus that specifically infects bacteria (**bacteriophage**). The tail is the portion of the bacteriophage that binds to the bacteria. Subsequently, the viral nucleic acid is injected into the bacterium via the tail. Details of the three-dimensional structure of the tail are crucial in designing ways to thwart the binding of the virus and the infection of the bacterium.

Proteins are also well suited to crystallography. The determination of the three-dimensional structure of proteins at a molecular level is necessary for the development of drugs that will be able to bind to the particular protein. Not surprisingly, the design of **antibiotics** relies heavily on protein crystallography.

The manufacture of a crystal of a protein species is not easy. Proteins tend to form three-dimensional structures that are quire irregular in shape because of the arrangement of the amino acid building blocks within the molecule. Some arrangements of the amino acids will produce flat sheets; other arrangements will result in a helix. Irregularly shaped molecules will not readily stack together with their counterparts. Moreover, once a crystal has formed, the structure is extremely fragile and can dissolve easily. This fragility does have an advantage, however, as it allows other molecules to be incorporated into the crystal during its formation. Thus, for example, an enzyme can become part of a crystal of its protein receptor, allowing the structure of the enzyme-receptor binding site to be studied.

A protein is crystallized by first making a very concentrated solution of the protein and then exposing the solution to chemicals that slowly increase the protein concentration. With the right combination of conditions the protein can spontaneously precipitate. The ideal situation is to have the precipitate begin at one site (the nucleation site). This site acts as the seed for more protein to come out of solution resulting in crystal formation.

Once a crystal has formed it must be delicately transferred to the machine where the x-ray diffraction will be performed. The crystal must be kept in an environment that maintains the crystal throughout the transfer of crystallographic procedures.

The entire process of protein crystallography is delicate and prone to error. Usually many failures occur before a successful experiment occurs. Yet, despite the effort and frustration, the information that can be obtained about protein structure is considerable.

See also Antibody-antigen, biochemical and molecular reactions; Biochemical analysis techniques; DNA (Deoxyribonucleic acid); Laboratory techniques in immunology; Laboratory techniques in microbiology; Molecular biology and molecular genetics; Proteins and enzymes; Vaccine

PROTEIN ELECTROPHORESIS • *see* ELECTROPHORESIS

Dr. Ergo Novotny examines a model of a protein.

PROTEIN EXPORT

Protein export is a process whereby protein that has been manufactured in a cell is routed to the surface of the cell. Export of proteins occurs in all **microorganisms**, but has been particularly well-studied in certain species of **bacteria** and **yeast**.

The ability of a cell to export protein is crucial to the survival or pathogenicity of the cell. Bacteria that have protein appendages for movement (e.g., flagella) and attachment (e.g., pili), and protective protein surface coatings (e.g., S layers) depend on the efficient export of the proteins. Exotoxins that are ultimately excreted by some bacteria need to get across the cell wall before being released from the bacterium.

Defects in protein export can produce or contribute to a number of maladies in eukaryotic cells including human cells (e.g., cystic fibrosis, diabetes, osteoporosis).

A general feature of protein export is the manufacture of a protein destined for secretion in a slightly longer form than the exported form of the protein. The additional stretch is known as the signal sequence, and its role in protein export forms the so-called **signal hypothesis**. Gunter Blobel garnered the 1999 Nobel Prize in Physiology or Medicine for his pioneering efforts in this area.

The "pre-protein" contains sequences of amino acids that give the precursor stretch of protein blocks that are hydrophilic (water-loving) and **hydrophobic** (water-hating). This allows a portion of the precursor region to spontaneously bury itself in the membrane layer that surrounds the interior of a bacterium, or the membrane of the endoplasmic reticulum of cells such as yeast. The hydrophilic sequences that flank the hydrophobic region associate with either side of the membrane. Thus, the precursor region is a membrane anchor.

Anchoring of the protein to the membrane is assisted by the action of two proteins. One of these proteins (designated SecB) associates with the precursor sequence of the newly made protein. The SecB protein then recognizes and binds to a protein called SecA that is embedded in the membrane. The SecB-SecA complex acts to guide the precursor region into position at the membrane. As the remainder of the protein is made, it is pushed out of the opposite side of the membrane. An enzyme associated with the outer surface of the membrane can snip off the precursor.

This process is sufficient for protein export in Gram-negative bacteria that have the single membrane. However, in Gram-negative bacteria the protein must be transported across the **periplasm** and the outer membrane before being truly exported. Furthermore, yeast cells require additional mecha-

nisms to route the protein from the Golgi apparatus to the exterior of the cell.

Proteins destined for export in Gram-negative bacteria are also synthesized as a precursor. The precursor functions at the outer membrane. Thus, the precursor must cross the inner membrane intact. This occurs because of an association that forms between a newly made precursor protein and a complex of several proteins. The protein complex is referred to as translocase. The translocase allows the precursor protein, with the hydrophobic region, to be completely transported across the inner membrane.

Studies using ***Escherichia coli*** and *Haemophilus influenzae* demonstrated the molecular nature of the translocase effect. The SecB protein is associated with the precursor region as a channel running alongside the precursor. The channel has a hydrophilic and a hydrophobic side. The latter is oriented outward so that it partitions into the hydrophobic interior of the bacterial inner membrane. The inner surface of SecB that is in intimate contact with the precursor region is hydrophilic. Thus, the precursor moves through the inner membrane in a watery channel.

As the precursor emerges into the periplasm, another protein present in the periplasm associates with the precursor region. This association also protects the precursor and allows the precursor to reach the inner surface of the outer membrane. Once there, the periplasmic protein is released, and the precursor sequence spontaneously inserts into the outer membrane.

Protein export has become an important target of strategies designed to thwart microorganism infections. By blocking the ability of certain proteins to be exported, the ability of bacteria to establish an infection can be hindered.

Conversely, the engineering of proteins to encourage their export can allow for the easier purification of commercially and clinically important proteins. For example, the engineering of human insulin in *Escherichia coli* relies on the export of the insulin protein. Once free of the bacteria, the insulin can be recovered in pure form much more easily and economically than if the protein needed to be extracted from the bacteria.

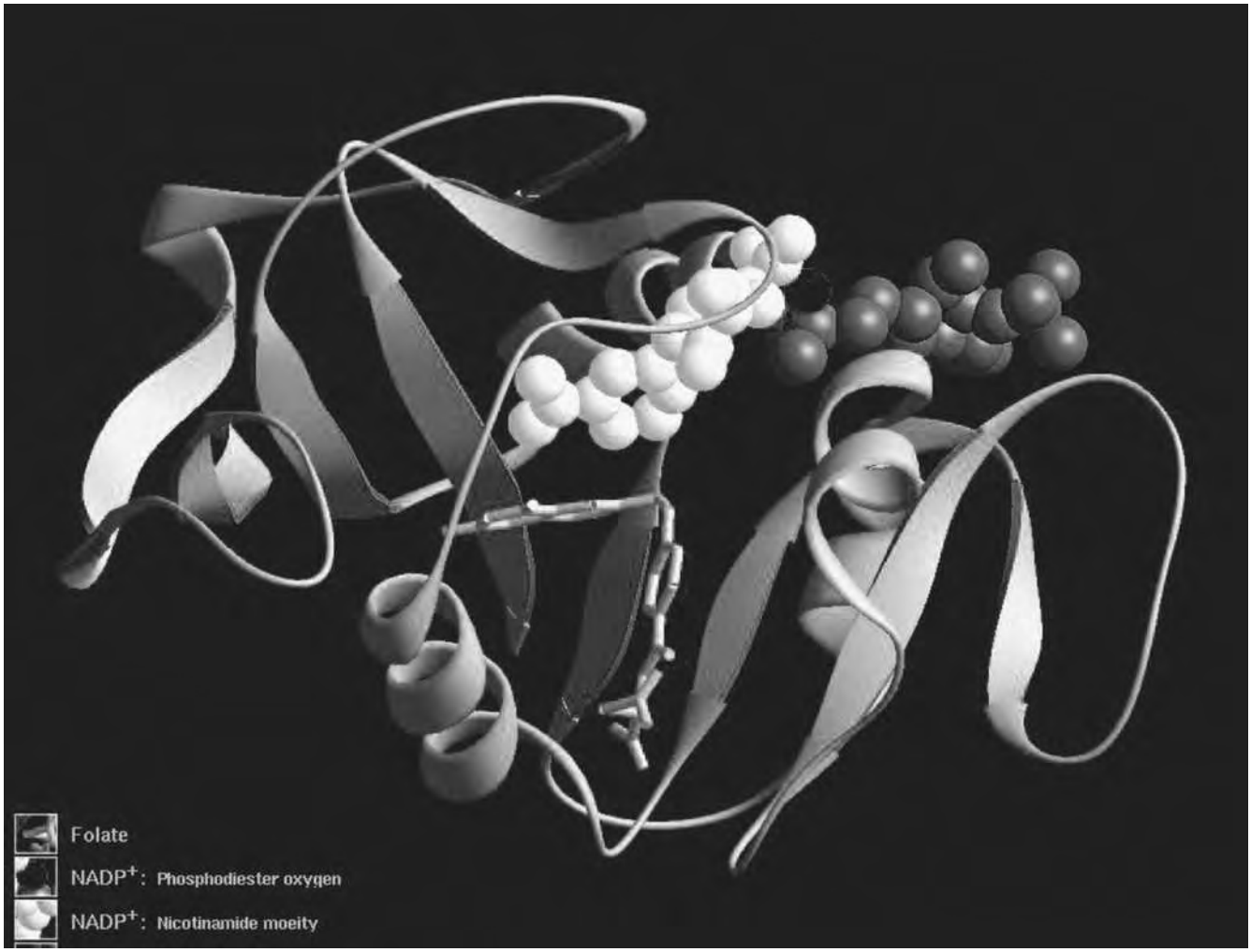
See also Bacteria and bacterial infection; Bacterial membranes and cell wall; Bacterial movement; Bacterial surface layers; Bacterial ultrastructure; Cell membrane transport; Enterotoxin and exotoxin; Molecular biology and molecular genetics; Prokaryotic membrane transport; Proteins and enzymes

PROTEIN SYNTHESIS

Protein synthesis represents the final stage in the **translation** of genetic information from **DNA**, via messenger **RNA** (mRNA), to protein. It can be viewed as a four-stage process, consisting of amino acid activation, translation initiation, chain elongation, and termination. The events are similar in both prokaryotes, such as **bacteria**, and higher eukaryotic organisms, although in the latter there are more factors involved in the process.

To begin with, each of the 20 cellular amino acids are combined chemically with a transfer RNA (tRNA) molecule to create a specific aminoacyl-tRNA for each amino acid. The process is catalyzed by a group of **enzymes** called aminoacyl-tRNA synthetases, which are highly specific with respect to the amino acid that they activate. The initiation of translation starts with the binding of the small subunit of a ribosome, (30S in prokaryotes, 40S in **eukaryotes**) to the initiation codon with the nucleotide sequence AUG, on the mRNA transcript. In prokaryotes, a sequence to the left of the AUG codon is recognized. This is the Shine-Delgrano sequence and is complementary to part of the small ribosome subunit. Eukaryotic **ribosomes** start with the AUG nearest the 5'-end of the mRNA, and recognize it by means of a "cap" of 7-methylguanosine triphosphate. After locating the cap, the small ribosome subunit moves along the mRNA until it meets the first AUG codon, where it combines with the large ribosomal subunit.

In both prokaryotes and eukaryotes, the initiation complex is prepared for the addition of the large ribosomal subunit at the AUG site, by the release of initiation factor (IF) 3. In bacteria, the large 50S ribosomal subunit appears simply to replace IF–3, with IF–1 and IF–2. In eukaryotes, another factor eIF–5 (eukaryotic initiation factor 5), catalyses the departure of the previous initiation factors and the joining of the large 60S ribosomal subunit. In both cases, the release of initiation factor 2 involves the hydrolysis of the GTP bound to it. At this stage, the first aminoacyl-tRNA, Met-tRNA, is bound to the ribosome. The ribosome can accommodate two tRNA molecules at once. One of these carries the Met-tRNA at initiation, or the peptide-tRNA complex during elongation and is thus called the P (peptide) site, while the other accepts incoming aminoacyl-tRNA and is therefore called the A (acceptor) site. What binds to the A site is usually a complex of GTP, elongation factor EF-TU, and aminoacyl-tRNA. The tRNA is aligned with the next codon on the mRNA, which is to be read and the elongation factor guides it to the correct nucleotide triplet. The energy providing GTP is then hydrolysed to GDP and the complex of EF-TU:GDP leaves the ribosome. The GDP is released from the complex when the EF-TU complexes with EF-TS, which is then replaced by GTP. The recycled EF-TU: GTP is then ready to pick up another aminoacyl-tRNA for addition to the growing polypeptide chain. On the ribosome, a reaction is catalysed between the carboxyl of the P site occupant and the free amino group of the A site occupant, linking the two together and promoting the growth of the polypeptide chain. The peptidyl transferase activity which catalyses this transfer is intrinsic to the ribosome. The final step of elongation is the movement of the ribosome relative to the mRNA accompanied by the translocation of the peptidyl-tRNA from the A to the P. Elongation factor EF-G is involved in this step and a complex of EF-G and GTP binds to the ribosome, GTP being hydrolysed in the course of the reaction. The de-acylated tRNA is also released at this time.



Computer image of a protein moleucle, showing regions of different three-dimensional shape.

The end of polypeptide synthesis is signalled by a termination codon contacting the A site. Three prokaryotic release factors (RF) are known: RF–1 is specific for termination codons UAA and UAG, while RF–2 is specific for UAA and UGA. RF–3 stimulates RF–1 and RF–2, but does not in itself recognize the termination codons. RF–3 also has GTPase activity and appears to accelerate the termination at the expense of GTP. Only one eukaryotic release factor is known and it has GTPase activity.

At any one time, there can be several ribosomes positioned along the mRNA and thus initiation, elongation and termination proceed simultaneously on the same length of mRNA. The three dimensional structure of the final protein begins to appear during protein synthesis before translation is completed. In many cases, after the synthesis of the amino acid chain, proteins are subjected to further reactions which convert them to their biologically active forms, e.g., by the attachment of chemical groups or by removal of certain amino acids—a processes known as post-translational modification.

See also Deoxyribonucleic acid (DNA); Genetic code; Molecular biology and molecular genetics; Ribonucleic acid (RNA)

PROTEINS AND ENZYMES

The building blocks of proteins and **enzymes** are molecules formed by carboxyl acids attached to amino groups ($–NH_2$), known as amino acids. Most protein structures consist of combinations of only about twenty of the most commonly found amino acids.

Amino acids bind to each other to form peptides and proteins. Conventionally, the term protein is used to designate chains of several peptides, known as polypeptides, with a molecular weight higher than thousands of Daltons. Peptides with a biological function go in length from dipeptides and tripeptides, up to polymers with thousands of Daltons.

Most proteins have well-defined structures and their specific biological functions depend upon the correct conforma-

Computer representation of the three-dimensional structure of a protein.

tion of the molecular structure. For instance, the majority of soluble proteins of an organism, such as blood proteins, have globular structures, like small eggs. Some proteins are fiber-like and are associated in bundles, forming fibrils such as those of wool and hair. Myosin, the protein that makes muscles contract, has both globular and fibrous elements in its structure; whereas collagen, the protein of connective tissues, is constituted by three triple helices of fibrils that form super structures in the shape of a fibrous rope. Collagen represents one third of all proteins of the human body and together with elastin is responsible for both cohesion and elasticity of tissues.

Every enzyme is also a protein. Enzymes are proteins that function as catalysts of biochemical reactions. Most physiological activities in organisms are mediated by enzymes, from unicellular life forms to mammals. Enzymes speed up chemical reactions, allowing organic systems to reach equilibrium in a faster pace. For instance, every phase of the **cell cycle** is controlled by enzymes that alternately inhibit or stimulate specific cellular activities as well as **gene** expression or repression, hence affecting the time of specific physiological activities within each phase of the cell cycle. Enzymes are highly selective in their activities, with each enzyme acting over a specific substrate or group of substrates. Substrate is a term designating any molecule that suffers enzymatic action, whether being activated or inhibited.

The main property of catalyst molecules is that they are not altered by the chemical reactions they induce, although some rare exceptions are known where some enzymes are inactivated by the reactions they catalyze. Enzymatic catalysis involves the formation of protein complexes between substrate and enzyme, where the amount of enzymes is generally much greater than the amount of substrate.

Some families of enzymes play an important role during the process of **DNA** replication. For example, when DNA synthesis activates, helicases break hydrogen bridges and some topoisomerases separate the two DNA strands. DNA-polymerases synthesize the fragments of the new DNA strand, while

topoisomerase III does the proofreading of the transcribed sequences, eliminating those containing errors. Ribonuclease H removes **RNA** sequences from polymers containing complexed RNA/DNA, and DNA-ligase unites the newly transcribed fragments, thus forming the new DNA strand.

In the last decade, researchers discovered that many proteins involved in intracellular communication are structured in a modular way. In other words, they are constituted by relatively short amino acid sequences of about 100 amino acids, and have the basic role of connecting one protein to another. Some proteins of such signaling pathways are entirely comprised of connecting modules and deprived of enzymatic activity. These non-enzymatic modules are termed protein dominium or protein modules, and they help enzymes in the transmission of signals to the cell **nucleus** in an orderly and controlled way. Proteins containing only connecting (or binding) modules, such as SH2 and SH3, act as important molecular adaptors to other proteins. While one of its modules binds to a signaling complex, such as a transmembrane tyrosine-kinase receptor, other binding modules permit the docking of other proteins that, once complexed, amplifies the signal to the nucleus. Such adaptor proteins also allow the cell to utilize certain enzymes that otherwise would not be activated in a given signaling pathway. The structure of adaptor proteins also displays binding sites that connect to DNA, where they recognize specific nucleotide sequences of a given gene, thus inducing **transcription**. In this case, the only enzyme in the cascade of signals to the nucleus is the receptor in the surface of the cell, and all the events that follow occur through the recognition among proteins and through the protein recognition of a locus in DNA.

Proteins are encoded by genes. A gene usually encodes a nucleotide sequence that can be first transcribed in pre-messenger RNA, and then read and translated on the **ribosomes** into a group of similar proteins with different lengths and functions, known as protein isoforms. A single polypeptide may be translated and then cut by enzymes into different proteins of variable lengths and molecular weights.

During transcription, the non-coding DNA sequences (introns) are cut off, and the coding sequences (exons) are transcribed into pre-messenger RNA, which in turn is spliced to a continuous stretch of exons before protein **translation** begins. The spliced stretch subdivides in codons, where any of the four kinds of nucleotide may occupy one or more of the three positions, and each triplet codes for one specific amino acid. The sequence of codons is read on the ribosomes, three nucleotides at a time. The order of codons determine the sequence of amino acids in the protein molecule that is formed.

Introns may have a regulatory role of either the splicing or the translational process, and may even serve as exons to other genes. After translation, proteins may also undergo biochemical changes, a process known as post-translation processing. They may be either cut by enzymes or receive special bonds, such as disulfide bridges, in order to fold into a functional structure.

See also Biochemistry; Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; DNA (Deoxyribonucleic acid); Transcription; Translation

PROTEOMICS

Proteomics is a discipline of microbiology and **molecular biology** that has arisen from the **gene** sequencing efforts that culminated in the sequencing of the human genome in the last years of the twentieth century. In addition to the human genome, sequences of disease-causing **bacteria** are being deduced. Although fundamental, knowledge of the sequence of nucleotides that comprise **deoxyribonucleic acid** reveals only a portion of the protein structure encoded by the **DNA**. Because proteins are an essential element of bacterial structure and function (e.g., role in causing infection), the knowledge of the three-dimensional structure and associations of proteins is vital. Proteomics is an approach to unravel the structure and function of proteins.

The word proteomics is derived from PROTEin **complement** to a genOME. Essentially, this is the spectrum of proteins that are produced from the template of an organism's genetic material under a given set of conditions. Proteomics compares the protein profiles of proteomes under different conditions in order to unravel biological processes.

The origin of proteomics dates back to the identification of the double-stranded structure of DNA by Watson and Crick in 1953. More recently, the development of the techniques of protein sequencing and gel **electrophoresis** in the 1960s and 1970s provided the technical means to probe protein structure. In 1986, the first protein sequence database was created (SWISS-PROT, located at the University of Geneva). By the mid-1990s, the concept of the proteome and the discipline of proteomics were well established. The power of proteomics was manifest in March 2000, when the complete proteome of a whole organism was published, that of the bacterium *Mycoplasma genitalium*

Proteomics research often involves the comparison of the proteins produced by a bacterium (example, ***Escherichia coli***) grown at different temperatures, or in the presence of different food sources, or a population grown in the lab versus a population recovered from an infection. *Escherichia coli* responds to changing environments by altering the proteins it produces. However, the full extent of the various alterations and their molecular bases are largely unknown. Proteomics research essentially attempts to provide a molecular explanation for bacterial behavior.

Proteomics can be widely applied to research of diverse microbes. For example, the **yeast** ***Saccharomyces cerevisiae*** is being studied to reveal the proteins produced and their functional associations with one another.

The task of sorting out all the proteins that can be produced by a bacterium or yeast cell is formidable. Targeting of the research effort is essential. For example, the comparison of the protein profile of a bacterium obtained directly from an infection (*in vivo*) with populations of the same microbe grown under defined conditions in the lab (*in vitro*) could

identify proteins that are unique to the infection. Some of these could become targets for diagnosis, therapy, or for prevention of the infection.

The study of proteins is difficult. The amount of protein cannot be amplified as easily as can the amount of DNA, making the detection of minute amounts of protein challenging. The structure of proteins must be maintained, which can be difficult. For example, **enzymes**, heat, light, or the energy of mixing can break down some proteins.

With the advent of the so-called **DNA chips**, the expression of thousands of genes can be monitored simultaneously. But DNA is static. It exists and is either expressed or not. Moreover, the expression of a protein does not necessarily mean that the protein is active. Also, proteins can be modified after being produced. Proteins can adopt different shapes, which can determine different functions and levels of activity after they have been produced. These functions provide the structural and operational framework for the life of the bacterium. Proteomics represents the next step after gene expression analysis

Proteomics utilizes various techniques to probe protein expression and structure. The migration of proteins can depend on their net charge and on the size of the protein molecule. When these migrations are in two dimensions, as in 2-D polyacrylamide gel electrophoresis, thousands of proteins can be distinguished in a single experiment. A technique called mass spectrometry analyzes a trait of proteins known as the mass-to-charge ratio, which essentially enables the sequence of amino acids comprising the protein to be determined. Techniques exist that detect modifications after protein manufacture, such as the addition of phosphate groups. Analogous to DNA chips, so-called protein microarrays have been developed. In these, a solid support holds various molecules (antibodies and receptors, as two examples) that will specifically bind protein. The binding pattern of proteins to the support can help determine what proteins are being made and when they are synthesized.

Proteomics typically operates in tandem with **bioinformatics**, which is an integration of mathematical, statistical, and computational methods to unravel biological data. The vast amount of protein information emerging from a single experiment would be impossible to analyze by manual computation or analysis. Accordingly, comparison of the data with other databases and the use of computer modeling programs, such as those that calculate three-dimensional structures, are invaluable in proteomics.

The knowledge of protein expression and structure, and the potential changes in structure and function under different conditions, could allow the tailoring of treatment strategies. For example, in the lungs of those afflicted with cystic fibrosis, the bacterium *Pseudomonas aeuruginosa* forms adherent populations on the surface of the lung tissue. These populations, which are enclosed in a **glycocalyx** that the bacteri produce, are very resistant to treatments and directly and indirectly damage the lung tissue to a lethal extent. Presently, it is known that the bacteria change their genetic expression as they become more firmly associated with the surface. Through proteomics, more details of the proteins involved in the initial approach to the surface and the subsequent, irreversible surface adhesion could be revealed. Once the targets are known, it is conceivable that they can be blocked. Thus, biofilms would not form and the bacteria could be more expeditiously eliminated from the lungs.

See also Biotechnology; Molecular biology and molecular genetics

PROTISTS

The kingdom Protista is the most diverse of all the five Eukaryotic kingdoms. There are more than 200,000 known species of protists with many more yet to be discovered. Protists can be found in countless colors, sizes, and shapes. They inhabit just about any area where water is found some or all of the time. They form the base of ecosystems by making food, as is the case with photosynthetic protists, or by themselves being eaten by larger organisms. They range in size from microscopic, unicellular organisms to huge seaweeds that can grow up to 300 ft (100 m) long.

The German zoologist Ernst Haeckel (1834–1919) first proposed the kingdom Protista in 1866. This early classification included any microorganism that was not a plant or an animal. Biologists did not readily accept this kingdom, and even after the American botanist Herbert F. Copeland again tried to establish its use 90 years later, there was not much support from the scientific community. Around 1960, R. Y. Stanier and **C. B. van Neil** (1897–1985) proposed the division of all organisms into two groups, the prokaryotes and the **eukaryotes**. Eukaryotes are organisms that have membrane-bound organelles in which metabolic processes take place, while prokaryotes lack these structures. In 1969, Robert Whittaker proposed the five-kingdom system of classification. The kingdom Protista was one of the five proposed kingdoms. At this time, only unicellular eukaryotic organisms were considered protists. Since then, the kingdom has expanded to include multicellular organisms, although biologists still disagree about what exactly makes an organism a protist.

Protists are difficult to characterize because of the great diversity of the kingdom. These organisms vary in body form, nutrition, and reproduction. They may be unicellular, colonial, or multicellular. As eukaryotes, protists can have many different organelles, including a **nucleus**, mitochondria, contractile vacuoles, food vacuoles, eyespots, plastids, pellicles, and flagella. The nuclei of protists contain **chromosomes**, with **DNA** associated with proteins. Protists are also capable of sexual, as well as asexual reproduction, meiosis, and mitosis. Protists can be free-living, or they may live symbiotically with another organism. This symbiosis can be mutualistic, where both partners benefit, or it can be parasitic, where the protist uses its host as a source of food or shelter while providing no advantage to the other organism. Many protists are economically important and beneficial to mankind, while others cause fatal diseases. Protists make up the majority of the **plankton** in aquatic systems, where they serve as the base of the food chain. Many protists are motile, using structures such as cilia, flagella, or

pseudopodia (false feet) to move, while others are sessile. They may be autotrophs, producing their own food from sunlight, or heterotrophs, requiring an outside source of nutrition. It is unknown whether protists were the precursors to plants, animals, or **fungi**. It is possible that several evolutionary lines of protists developed separately. Biologists consider the protists as a polyphyletic group, meaning they probably do not share a common ancestor. The word protist comes from the Greek word for the very first, which indicates that researchers assume protists may have been the first eukaryotes to evolve on Earth.

Despite the great diversity evident in this kingdom, scientists have been able to classify the protists into several groups. The protists can be classified into one of three main categories, animal-like, plant-like, and fungus-like. Grouping into one of the three categories is based on an organism's mode of reproduction, method of nutrition, and motility. The animal-like protists are known as the **protozoa**, the plant-like protists are the algae, and the fungus-like protists are the **slime molds** and water molds.

The protozoa are all unicellular heterotrophs. They obtain their nutrition by ingesting other organisms or dead organic material. The word protozoa comes from the Latin word for first animals. The protozoans are grouped into various phyla based on their modes of locomotion. They may use cilia, flagella, or pseudopodia. Some protozoans are sessile, meaning they do not move. These organisms are parasitic because they cannot actively capture food. They must live in an area of the host organism that has a constant food supply, such as the intestines or bloodstream of an animal. The protozoans that use pseudopodia to move are known as amoebas, those that use flagella are called flagellates, those that use cilia are known as the ciliates, and those that do not move are called the sporozoans.

The amoebas belong to the phylum Rhizopoda. These protists have no wall outside of their cell membrane. This gives the cell flexibility and allows it to change shape. The word amoeba, in fact, comes from the Greek word for change. Amoebas use extensions of their cell membrane, called pseudopodia, to move as well as to engulf food. When the pseudopodium traps a bit of food, the cell membrane closes around the meal. This encasement forms a food vacuole. Digestive **enzymes** are secreted into the food vacuole, which break down the food. The cell then absorbs the nutrients. Because amoebas live in water, dissolved nutrients from the environment can diffuse directly through their cell membranes. Most amoebas live in marine environments, although some freshwater species exist. Freshwater amoebas live in a hypotonic environment, so water is constantly moving into the cell by osmosis. To remedy this problem, these amoebas use contractile vacuoles to pump excess water out of the cell. Most amoebas reproduce asexually by pinching off a part of the cell membrane to form a new organism. Amoebas may form cysts when environmental conditions become unfavorable. These cysts can survive conditions such as lack of water or nutrients. Two forms of amoebas have shells, the foraminiferans and the radiolarians.

The foraminiferans have a hard shell made of calcium carbonate. These shells are called tests. Foraminiferans live in marine environments and are very abundant. When they die, their shells fall to the ground where they become a part of the muddy ocean floor. Geologists use the fossilized shells to determine the ages of rocks and sediments. The shells at the ocean floor are gradually converted into chalky deposits, which can be uplifted to become a land formation, such as the white cliffs of Dover in England. Radiolarians have shells made of silica instead of calcium carbonate. Both organisms have many tiny holes in their shells, through which they extend their pseudopodia. The pseudopodia act as a sticky net, trapping bits of food.

The flagellates have one or more flagella and belong to the phylum Zoomastigina. These organisms whip their flagella from side to side in order to move through their aquatic surroundings. These organisms are also known as the zooflagellates. The flagellates are mostly unicellular with a spherical or oblong shape. A few are also amoeboid. Many ingest their food through a primitive mouth, called the oral groove. While most are motile, one class of flagellates, called the Choanoflagellates, is sessile. These organisms attach to a rock or other substrate by a stalk.

The ciliates are members of the phylum Ciliopa. There are approximately 8,000 species of ciliates. These organisms move by the synchronized beating of the cilia covering their bodies. They can be found almost anywhere, in freshwater or marine environments. Probably the best-known ciliate is the organism **Paramecium**. Paramecia have many well-developed organelles. Food enters the cell through the oral groove (lined with cilia, to "sweep" the food into the cell), where it moves to the gullet, which packages the meal into a food vacuole. Enzymes released into the food vacuole break down the food, and the nutrients are absorbed into the cell. Wastes are removed from the cell through an anal pore. Contractile vacuoles pump out excess water, since paramecia live in freshwater (hypotonic) surroundings. Paramecia have two nuclei, a macronucleus and a micronucleus. The larger macronucleus controls most of the metabolic functions of the cell. The smaller micronucleus controls much of the pathways involved in sexual reproduction. Thousands of cilia appear through the pellicle, a tough, protective covering surrounding the cell membrane. These cilia beat in a synchronized fashion to move the Paramecium in any direction. Underneath the pellicle are trichocysts, which discharge tiny spikes that help trap prey. Paramecia usually reproduce asexually, when the cell divides into two new organisms after all of the organelles have been duplicated. When conditions are unfavorable, however, the organism can reproduce sexually. This form of sexual reproduction is called **conjugation**. During conjugation, two paramecia join at the oral groove, where they exchange genetic material. They then separate and divide asexually, although this division does not necessarily occur immediately following the exchange of genetic material.

The sporozoans belong to the phylum **Sporozoa**. These organisms are sessile, so they cannot capture prey. Therefore, the sporozoans are all **parasites**. As their name suggests, many of these organisms produce spores, reproductive cells that can give rise to a new organism. Sporozoans typically have com-

plex life cycles, as they usually live in more than one host in their lifetimes.

The plant-like protists, or algae, are all photosynthetic autotrophs. These organisms form the base of many food chains. Other creatures depend on these protists either directly for food or indirectly for the oxygen they produce. Algae are responsible for over half of the oxygen produced by photosynthesizing organisms. Many forms of algae look like plants, but they differ in many ways. Algae do not have roots, stems, or leaves. They do not have the waxy cuticle plants have to prevent water loss. As a result, algae must live in areas where water is readily available. Algae do not have multicellular gametangia as the plants do. They contain **chlorophyll**, but also contain other photosynthetic pigments. These pigments give the algae characteristic colors and are used to classify algae into various phyla. Other characteristics used to classify algae are energy reserve storage and cell wall composition.

Members of the phylum Euglenophyta are known as euglenoids. These organisms are both autotrophic as well as heterotrophic. There are hundreds of species of euglenoids. Euglenoids are unicellular and share properties of both plants and animals. They are plant-like in that they contain chlorophyll and are capable of **photosynthesis**. They do not have a cell wall of cellulose, as do plants; instead, they have a pellicle made of protein. Euglenoids are like animals in that they are motile and responsive to outside stimuli. One particular species, Euglena, has a structure called an eyespot. This area of red pigments is sensitive to light. An Euglena can respond to its environment by moving towards areas of bright light, where photosynthesis best occurs. In conditions where light is not available for photosynthesis, euglenoids can be heterotrophic and ingest their food. Euglenoids store their energy as paramylon, a type of polysaccharide.

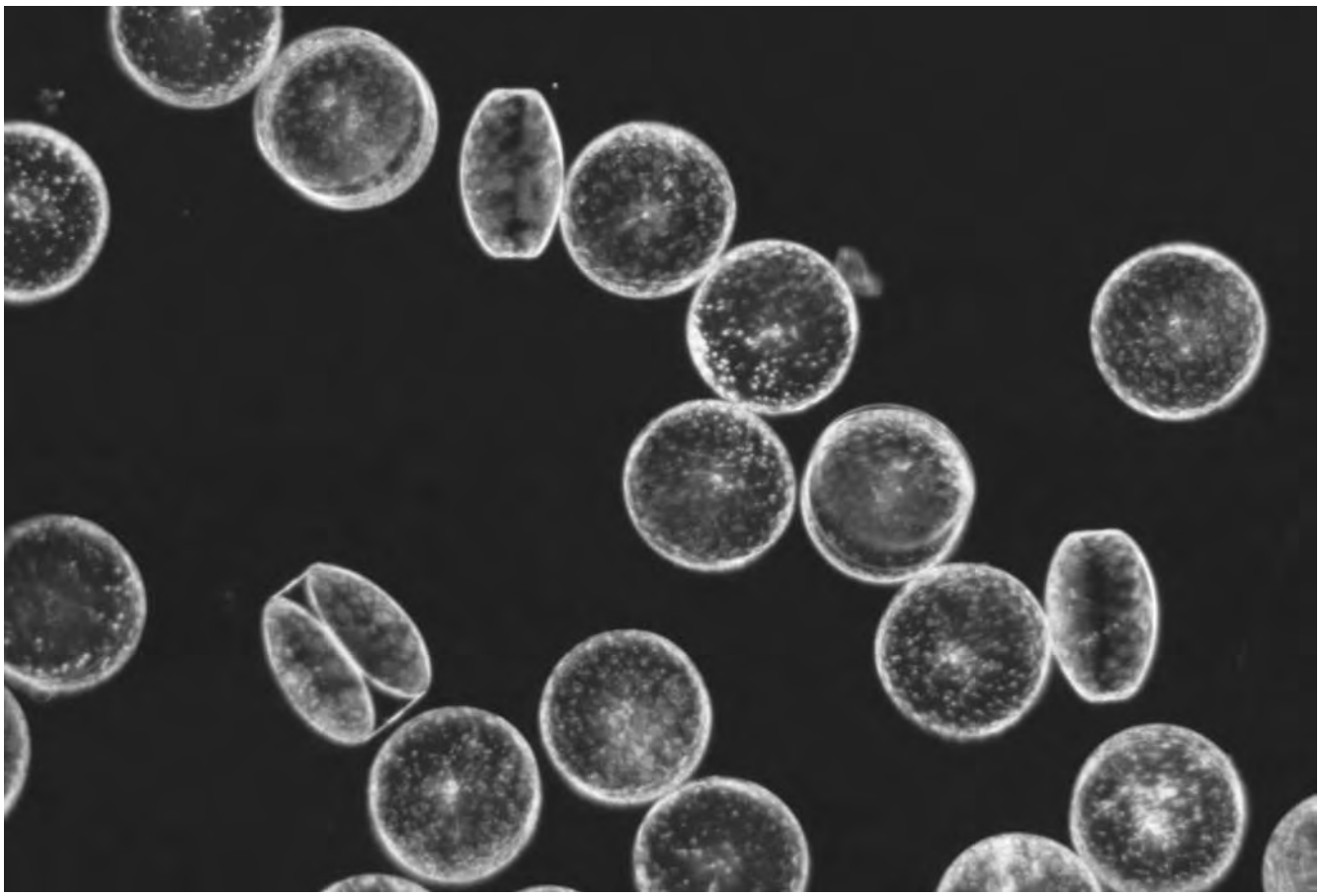
Members of the phylum Bacillariophyta are called **diatoms**. Diatoms are unicellular organisms with silica shells. They are autotrophs and can live in marine or freshwater environments. They contain chlorophyll as well as pigments called carotenoids, which give them an orange-yellow color. Their shells resemble small boxes with lids. These shells are covered with grooves and pores, giving them a decorated appearance. Diatoms can be either radially or bilaterally symmetrical. Diatoms reproduce asexually in an unique manner. The two halves of the shell separate, each producing a new shell that fits inside the original half. Each new generation, therefore, produces offspring that are smaller than the parent. As each generation gets smaller and smaller, a lower limit is reached, approximately one quarter the original size. At this point, the diatom produces gametes that fuse with gametes from other diatoms to produce zygotes. The zygotes develop into full sized diatoms that can begin asexual reproduction once more. When diatoms die, their shells fall to the bottom of the ocean and form deposits called diatomaceous earth. These deposits can be collected and used as abrasives, or used as an additive to give certain paints their sparkle. Diatoms store their energy as oils or carbohydrates.

The **dinoflagellates** are members of the phylum Dinoflagellata. These organisms are unicellular autotrophs. Their cell walls contain cellulose, creating thick, protective plates. These plates contain two grooves at right angles to each other, each groove containing one flagellum. When the two flagella beat together, they cause the organism to spin through the water. Most dinoflagellates are marine organisms, although some have been found in freshwater environments. Dinoflagellates contain chlorophyll as well as carotenoids and red pigments. They can be free-living, or live in symbiotic relationships with jellyfish or corals. Some of the free-living dinoflagellates are bioluminescent. Many dinoflagellates produce strong toxins. One species in particular, Gonyaulax catanella, produces a lethal nerve toxin. These organisms sometimes reproduce in huge amounts in the summertime, causing a **red tide**. There are so many of these organisms present during a red tide that the ocean actually appears red. When this occurs, the toxins that are released reach such high concentrations in the ocean that many fish are killed. Dinoflagellates store their energy as oils or polysaccharides.

The phylum **Rhodophyta** consists of the red algae. All of the 4,000 species in this phylum are multicellular (with the exception of a few unicellular species) and live in marine environments. Red algae are typically found in tropical waters and sometimes along the coasts in cooler areas. They live attached to rocks by a structure called a holdfast. Their cell walls contain thick polysaccharides. Some species incorporate calcium carbonate from the ocean into their cell walls as well. Red algae contain chlorophyll as well as phycobilins, red and blue pigments involved in photosynthesis. The red pigment is called phycoerythrin and the blue pigment is called phycocyanin. Phycobilins absorb the green, violet, and blue light waves that can penetrate deep water. These pigments allow the red algae to photosynthesize in deep water with little light available. Reproduction in these organisms is a complex alternation between sexual and asexual phases. Red algae store their energy as floridean starch.

The 1,500 species of brown algae are the members of the phylum Phaeophyta. The majority of the brown algae live in marine environments, on rocks in cool waters. They contain chlorophyll as well as a yellow-brown carotenoid called fucoxanthin. The largest of the brown algae are the **kelp**. The kelp use holdfasts to attach to rocks. The body of a kelp is called a thallus, which can grow as long as 180 ft (60 m). The thallus is composed of three sections, the holdfast, the stipe, and the blade. Some species of brown algae have an air bladder to keep the thallus floating at the surface of the water, where more light is available for photosynthesis. Brown algae store their energy as laminarin, a carbohydrate.

The phylum **Chlorophyta** is known as the green algae. This phylum is the most diverse of all the algae, with greater than 7,000 species. The green algae contain chlorophyll as their main pigment. Most live in fresh water, although some marine species exist. Their cell walls are composed of cellulose, which indicates the green algae may be the ancestors of modern plants. Green algae can be unicellular, colonial, or multicellular. An example of a unicellular green alga is Chlamydomonas. An example of a colonial algae is Volvox. A Volvox **colony** is a hollow sphere of thousands of individual cells. Each cell has a single flagellum that faces the exterior of the sphere. The individual cells beat their flagella in a coordi-



Diatoms, an example of protists.

nated fashion, allowing the colony to move. Daughter colonies form inside the sphere, growing until they reach a certain size and are released when the parent colony breaks open. Spirogyra and Ulva are both examples of multicellular green algae. Reproduction in the green algae can be both sexual and asexual. Green algae store their energy as starch.

The fungus-like protists resemble the fungi during some part of their life cycle. These organisms exhibit properties of both fungi and protists. The slime molds and the water molds are members of this group. They all obtain energy by decomposing organic materials, and as a result, are important for recycling nutrients. They can be brightly colored and live in cool, moist, dark habitats. The slime molds are classified as either plasmodial or cellular by their modes of reproduction. The plasmodial slime molds belong to the phylum Myxomycota, and the cellular slime molds belong to the phylum Acrasiomycota.

The plasmodial slime molds form a structure called a **plasmodium**, a mass of **cytoplasm** that contains many nuclei but has no cell walls or membranes to separate individual cells. The plasmodium is the feeding stage of the slime **mold**. It moves much like an amoeba, slowly sneaking along decaying organic material. It moves at a rate of 1 in (2.5 cm) per hour, engulfing **microorganisms**. The reproductive structure of plasmodial slime molds occurs when the plasmodium forms a stalked structure during unfavorable conditions. This structure produces spores that can be released and travel large distances. The spores land and produce a zygote that grows into a new plasmodium.

The cellular slime molds exist as individual cells during the feeding stage. These cells can move like an amoeba as well, engulfing food along the way. The feeding cells reproduce asexually through cell division. When conditions become unfavorable, the cells come together to form a large mass of cells resembling a plasmodium. This mass of cells can move as one organism and looks much like a garden slug. The mass eventually develops into a stalked structure capable of sexual reproduction.

The water molds and downy mildews belong to the phylum Oomycota. They grow on the surface of dead organisms or plants, decomposing the organic material and absorbing nutrients. Most live in water or in moist areas. Water molds grow as a mass of fuzzy white threads on dead material. The difference between these organisms and true fungi is the water molds form flagellated reproductive cells during their life cycles.

Many protists can cause serious illness and disease. **Malaria**, for example, is caused by the protist Plasmodium. Plasmodia are sporozoans and are transferred from person to

person through female Anopheles mosquitoes. People who suffer from malaria experience symptoms such as shivering, sweating, high fevers, and delirium. African **sleeping sickness**, also known as African trypanosomiasis, is caused by another sporozoan, Trypanosoma. Trypanosoma is transmitted through the African tsetse fly. This organism causes high fever and swollen lymph nodes. Eventually the protist makes its way into the victim's brain, where it causes a feeling of uncontrollable fatigue. Giardiasis is another example of a disease caused by a protist. This illness is caused by **Giardia**, a sporozoan carried by muskrats and beavers. Giardiasis is characterized by fatigue, cramps, diarrhea, and weight loss. Amoebic **dysentery** occurs when a certain amoeba, *Entamoeba histolytica,* infects the large intestine of humans. It is spread through infected food and water. This organism causes bleeding, diarrhea, vomiting, and sometimes death.

Members of the kingdom Protista can also be very beneficial to life on Earth. Many species of red algae are edible and are popular foods in certain parts of the world. Red algae are rich in vitamins and minerals. Carageenan, a polysaccharide extracted from red algae, is used as a thickening agent in ice cream and other foods. Giant kelp forests are rich ecosystems, providing food and shelter for many organisms. Trichonymphs are flagellates that live in the intestines of termites. These protozoans break down cellulose in wood into carbohydrates the termites can digest.

The kingdom Protista is a diverse group of organisms. Some protists are harmful, but many more are beneficial. These organisms form the foundation for food chains, produce the oxygen we breathe, and play an important role in nutrient recycling. Many protists are economically useful as well. As many more of these unique organisms are discovered, humans will certainly enjoy the new uses and benefits protists provide.

See also Eukaryotes

PROTOPLASTS AND SPHEROPLASTS

Protoplasts and spheroplasts are altered forms of **bacteria** or **yeast**, in which the principal shape-maintaining structure of the bacteria is weakened. Each bacterium forms a sphere, which is the shape that allows the bacterium to withstand the rigors, particularly osmotic, of the fluid in which it resides.

The term protoplast refers to the spherical shape assumed by Gram-positive bacteria. Spheroplast refers to the spherical shape assumed by Gram-negative bacteria. The difference is essentially the presence of a single membrane, in the case of the protoplast, and the two membranes (inner and outer) of the Gram-negative spheroplasts. It is also possible to generate a gram-negative protoplast by the removal of the outer membrane. Thus, in essence, protoplast refers to a bacterial sphere that is bounded by a single membrane and spheroplast refers to a sphere that is bounded by two membranes.

Bacteria are induced to form protoplasts or spheroplasts typically by laboratory manipulation. However, formation of the structures can occur naturally. Such bacteria are referred to as L-forms. Examples of bacterial genera that can produce L-forms include *Bacillus*, *Clostridium*, *Haemophilus*, *Pseudomonas*, *Staphylococcus*, and *Vibrio*.

The **peptidoglycan** is the main stress-bearing layer of the bacterial cell wall and the peptidoglycan also gives the bacterium its shape. In the laboratory, weakening the peptidoglycan network in the cell wall generates both protoplasts and spheroplasts.

By exposing bacteria to an enzyme called lysozyme, the interconnecting strands of the two particular sugars that form the peptidoglycan can be cut. When this is done, the peptidoglycan loses the ability to serve as a mechanical means of support.

The situation in yeast is slightly different, as other components of the yeast cell wall are degraded in order to form the protoplast.

The process of creating protoplasts and spheroplasts must be done in a solution in which the ionic composition and concentration of the fluid outside of the bacteria is the same as that inside the bacteria. Once the structural support of the peptidoglycan is lost, the bacteria are unable to control their response to differences in the ionic composition between the bacterial interior and exterior. If the inner concentration is greater than the outer ionic concentration, water will flow into the bacterium in an attempt to achieve an ionic balance. The increased volume can be so severe that the bacteria will burst. Conversely, if the inner ionic concentration is less than the exterior, water will exit the bacterium, in an attempt to dilute the surroundings. The bacteria can shrivel to the point of death.

Preservation of ionic balance is required to ensure that bacteria will not be killed during their **transformation** into either the protoplast or the spheroplast form. Living protoplasts and spheroplasts are valuable research tools. The membrane balls that are the protoplasts or spheroplasts can be induced to fuse more easily with similar structures as well as with eukaryotic cells. This facilitates the transfer of genetic material between the two cells. As well, the sequential manufacture of spheroplasts and protoplasts in Gram-negative bacteria allows for the selective release of the contents of the **periplasm**. This approach has been popular in the identification of the components of the periplasm, and in the localization of proteins to one or the other of the Gram-negative membranes. For example, if a certain protein is present in a spheroplast population—but is absent from a protoplast population—then the protein is located within the outer membrane.

See also Bacterial ultrastructure; Biotechnology; Transformation

PROTOZOA

Protozoa are a very diverse group of single-celled organisms, with more than 50,000 different types represented. The vast majority are microscopic, many measuring less than 1/200 mm, but some, such as the freshwater Spirostomun, may reach 0.17 in (3 mm) in length, large enough to enable it to be seen with the naked eye.

Scientists have discovered fossilized specimen of protozoa that measured 0.78 in (20 mm) in diameter. Whatever the size, however, protozoans are well-known for their diversity and the fact that they have evolved under so many different conditions.

One of the basic requirements of all protozoans is the presence of water, but within this limitation, they may live in the sea, in rivers, lakes, stagnant ponds of freshwater, soil, and in some decaying matters. Many are solitary organisms, but some live in colonies; some are free-living, others are sessile; and some species are even **parasites** of plants and animals (including humans). Many protozoans form complex, exquisite shapes and their beauty is often greatly overlooked on account of their diminutive size.

The protozoan cell body is often bounded by a thin pliable membrane, although some sessile forms may have a toughened outer layer formed of cellulose, or even distinct shells formed from a mixture of materials. All the processes of life take place within this cell wall. The inside of the membrane is filled with a fluid-like material called **cytoplasm**, in which a number of tiny organs float. The most important of these is the **nucleus**, which is essential for growth and reproduction. Also present are one or more contractile vacuoles, which resemble air bubbles, whose job it is to maintain the correct water balance of the cytoplasm and also to assist with food assimilation.

Protozoans living in salt water do not require contractile vacuoles as the concentration of salts in the cytoplasm is similar to that of seawater and there is therefore no net loss or gain of fluids. Food vacuoles develop whenever food is ingested and shrink as digestion progresses. If too much water enters the cell, these vacuoles swell, move towards the edge of the cell wall and release the water through a tiny pore in the membrane.

Some protozoans contain the green pigment **chlorophyll** more commonly associated with higher plants, and are able to manufacture their own foodstuffs in a similar manner to plants. Others feed by engulfing small particles of plant or animal matter. To assist with capturing prey, many protozoans have developed an ability to move. Some, such as Euglena and Trypanosoma are equipped with a single whip like flagella which, when quickly moved back and forth, pushes the body through the surrounding water body. Other protozoans (e.g., Paramecium) have developed large numbers of tiny cilia around the membrane; the rhythmic beat of these hairlike structures propel the cell along and also carry food, such as **bacteria**, towards the gullet. Still others are capable of changing the shape of their cell wall. The Amoeba, for example, is capable of detecting chemicals given off by potential food particles such as **diatoms**, algae, bacteria or other protozoa. As the cell wall has no definite shape, the cytoplasm can extrude to form pseudopodia (Greek *pseudes*, "false"; *pous*, "foot") in various sizes and at any point of the cell surface. As the Amoeba approaches its prey, two pseudopodia extend out from the main cell and encircle and engulf the food, which is then slowly digested.

Various forms of reproduction have evolved in this group, one of the simplest involves a splitting of the cell in a process known as binary fission. In species like amoeba, this process takes place over a period of about one hour: the nucleus divides and the two sections drift apart to opposite ends of the cell. The cytoplasm also begins to divide and the cell changes shape to a dumb-bell appearance. Eventually the cell splits giving rise to two identical "daughter" cells that then resume moving and feeding. They, in turn, can divide further in this process known as asexual reproduction, where only one individual is involved.

Some species that normally reproduce asexually, may occasionally reproduce through sexual means, which involves the joining, or fusion, of the nuclei from two different cells. In the case of **paramecium**, each individual has two nuclei: a larger macronucleus that is responsible for growth, and a much smaller micronucleus that controls reproduction. When paramecium reproduce by sexual means, two individuals join in the region of the oral groove—a shallow groove in the cell membrane that opens to the outside. When this has taken place, the macronuclei of each begins to disintegrate, while the micronucleus divides in four. Three of these then degenerate and the remaining nucleus divides once again to produce two micronuclei that are genetically identical. The two cells then exchange one of these nuclei that, upon reaching the other individual's micronucleus, fuse to form what is known as a zygote nucleus. Shortly afterwards, the two cells separate but within each cell a number of other cellular and cytoplasmic divisions will continue to take place, eventually resulting in the production of four daughter cells from each individual.

Protozoans have evolved to live under a great range of environmental conditions. When these conditions are unfavorable, such as when food is scarce, most species are able to enter an inactive phase, where cells become non-motile and secrete a surrounding cyst that prevents **desiccation** and protects the cell from extreme temperatures. The cysts may also serve as a useful means of dispersal, with cells being borne on the wind or on the feet of animals. Once the cyst reaches a more favorable situation, the outer wall breaks down and the cell resumes normal activity.

Many species are of considerable interest to scientists, not least because of the medical problems that many cause. The tiny **Plasmodium** protozoan, the cause of **malaria** in humans, is responsible for hundreds of millions of cases of illness each year, with many deaths occurring in poor countries. This parasite is transferred from a malarial patient to a healthy person by the bite of female mosquitoes of the genus Anopheles. As the mosquito feeds on a victim's blood the parasites pass from its salivary glands into the open wound. From there, they make their way to the liver where they multiply and later enter directly into red blood cells. Here they multiply even further, eventually causing the blood cell to burst and release from 6-36 infectious bodies into the blood plasma. A mosquito feeding on such a patient's blood may absorb some of these organisms, allowing the parasite to complete its life cycle and begin the process all over again. The shock of the release of so many parasites into the human blood stream results in a series of chills and fevers—typical symptoms of malaria. Acute cases of malaria may continue for some days or even weeks, and may subside if the body is able to develop **immunity** to the disease. Relapses, however, are common and

malaria is still a major cause of death in the tropics. Although certain drugs have been developed to protect people from Plasmodium many forms of malaria have now developed, some of which are even immune to the strongest medicines.

While malaria is one of the best known diseases known to be caused by protozoans, a wide range of other equally devastating ailments are also caused by protozoan infections. Amoebic **dysentery**, for example, is caused by *Entamoeba histolytica*.; African **sleeping sickness**, which is spread by the bite of the tsetse fly, is caused by the flagellate protozoan Trypanosoma; a related species *T. cruzi* causes Chagas' disease in South and Central America; Eimeria causes coccidiosis in rabbits and poultry; and Babesia, spread by ticks, causes red water fever in cattle.

Not all protozoans are parasites however, although this is by far a more specialized life style than that adopted by free-living forms. Several protozoans form a unique, nondestructive, relationship with other species, such as those found in the intestine of wood-eating termites. Living in the termites' intestines the protozoans are provided with free board and lodgings as they ingest the wood fibers for their own nutrition. In the process of doing so, they also release proteins which can be absorbed by the termite's digestive system, which is otherwise unable to break down the tough cellulose walls of the wood fibers. Through this mutualistic relationship, the termites benefit from a nutritional source that they could otherwise not digest, while the protozoans receive a safe home and steady supply of food.

See also Amoebic dysentery; Entamoeba histolytica; Epidemiology, tracking diseases with technology; Waste water treatment; Water quality

PRUSINER, STANLEY (1942-)

American physician

Stanley Prusiner performed seminal research in the field of neurogenetics, identifying the prion, a unique infectious protein agent containing no **DNA** or **RNA**.

Prusiner was born on in Des Moines, Iowa. His father, Lawrence, served in the United States Navy, moving the family briefly to Boston where Lawrence Prusiner enrolled in Naval officer training school before being sent to the South Pacific. During his father's absence, the young Stanley lived with his mother in Cincinnati, Ohio. Shortly after the end of World War II, the family returned to Des Moines where Stanley attended primary school and where his brother, Paul, was born. In 1952, the family returned to Ohio where Lawrence Prusiner worked as a successful architect.

In Ohio, Prusiner attended the Walnut Hills High School, before being accepted by the University of Pennsylvania where he majored in Chemistry. At the University, besides numerous science courses, he also had the opportunity to broaden his studies in subjects such as philosophy, the history of architecture, economics, and Russian history. During the summer of 1963, between his junior and senior years, he began a research project on hypothermia with Sidnez Wolfson in the Department of Surgery. He worked on the project throughout his senior year and then decided to stay on at the University to train for medical school. During his second year of medicine, Prusiner decided to study the surface fluorescence of brown adipose tissue (fatty tissue) in Syrian golden hamsters as they arose from hibernation. This research allowed him to spend much of his fourth study year at the Wenner-Gren Institute in Stockholm working on the **metabolism** of isolated brown adipocytes. At this he began to seriously consider pursuing a career in biomedical research.

Early in 1968, Prusiner returned to the U.S. to complete his medical studies. The previous spring, he had been given a position at the National Institutes of Health (NIH) on completing an internship in medicine at the University of California San Francisco (UCSF). During that year, he met his wife, Sandy Turk, who was teaching mathematics to high school students. At the NIH, he worked on the glutaminase family of **enzymes** in ***Escherichia coli*** and as the end of his time at the NIH began to near, he examined the possibility of taking up a postdoctoral fellowships in neurobiology. Eventually, however, he decided that a residency in neurology was a better route to developing a rewarding career in research as it offered him direct contact with patients and therefore an opportunity to learn about both the normal and abnormal nervous system. In July 1972, Prusiner began a residency at UCSF in the Department of Neurology. Two months later, he admitted a female patient who was exhibiting progressive loss of memory and difficulty performing some routine tasks. This was his first encounter with a Creutzfeldt-Jakob disease (CJD) patient and was the beginning of the work to which he has dedicated most of his life.

In 1974, Prusiner accepted the offer of an assistant professor position from Robert Fishman, the Chair of Neurology at UCSF, and began to set up a laboratory to study scrapie, a parallel disease of human CJD found in sheep. Early on in this endeavor, he collaborated with William Hadlow and Carl Eklund at the Rocky Mountain Laboratory in Hamilton, Montana, from whom he learnt much about the techniques of handling the scrapie agent. Although the agent was first believed to be a virus, data from the very beginning suggested that this was a novel infectious agent, which contained no nucleic acid. It confirmed the conclusions of Tikvah Alper and J. S. Griffith who had originally proposed the idea of an infectious protein in the 1960s. The idea had been given little credence at that time. At the beginning of his research into prion diseases, Prusiner's work was fraught with technical difficulties and he had to stand up to the skepticism of his colleagues. Eventually he was informed by the Howard Hughes Medical Institute (HHMI) that they would not renew their financial support and by UCSF that he would not be promoted to tenure. The tenure decision was eventually reversed, however, enabling Prusiner to continue his work with financial support from other sources. As the data for the protein nature of the scrapie agent accumulated, Prusiner grew more confident that his findings were not artifacts and decided to summarize his work in a paper, published in 1982. There he introduced the term "prion," derived from "proteinaceous" and 'infectious" particle and challenged the scientific community to attempt to

find an associated nucleic acid. Despite the strong convictions of many, none was ever found.

In 1983, the protein of the prion was found in Prusiner's laboratory and the following year, a portion of the amino acid sequence was determined by Leroy Hood. With that knowledge, molecular biological studies of **prions** ensued and an explosion of new information followed. Prusiner collaborated with Charles Weissmann on the molecular **cloning** of the **gene** encoding the prion protein (PrP). Work was also done on linking the PrP gene to the control of scrapie incubation times in mice and on the discovery that **mutations** within the protein itself caused different incubation times. Antibodies that provided an extremely valuable tool for prion research were first raised in Prusiner's lab and used in the discovery of the normal form of PrP protein. By the early 1990s, the existence of prions as causative agents of diseases like CJD in humans and bovine spongiform encephalopathy (BSE) in cows, came to be accepted in many quarters of the scientific community. As prions gained wider acceptance among scientists, Prusiner received many scientific prizes. In 1997, Prusiner was awarded the Nobel Prize for medicine.

See also BSE and CJD disease; Infection and resistance; Viral genetics

Pseudomembranous colitis

Pseudomembranous colitis is severe **inflammation** of the colon in which raised, yellowish plaques, or pseudomembranes, develop on the mucosal lining. The plaques consist of clumps of dead epithelial cells from the colon, white blood cells, and fibrous protein.

Pseudomembranous colitis is usually associated with antibiotic use. When the normal balance of the flora in the colon is disturbed, pathogenic strains of the bacillus *Clostridium difficile* may proliferate out of control and produce damaging amounts of cytotoxins known as cytotoxins A and B.

C. difficile toxins often cause diarrhea and mild inflammation of the colon. Less frequently, the condition may progress further, causing ulceration and formation of the pseudomembranous plaques. Pseudomembranous colitis is most common in health care facilities such as hospitals and nursing homes, where an individual is most likely to be immune-compromised and to come into contact with persistent, heat-resistant *C. difficile* spores by the fecal-oral route. Thus, the best way to prevent it is meticulous cleanliness, coupled with avoiding the overuse of **antibiotics**.

Mild symptoms such as diarrhea often disappear spontaneously soon after the antibiotics are discontinued. Ironically, severe antibiotic-associated colitis must generally be treated with additional antibiotics to target the *C. difficile* pathogen. Benign intestinal flora such as **lactobacillus** or nonpathogenic **yeast** may be administered orally or rectally. Supportive therapies such as intravenous fluids are used as in other cases of ulcerative colitis. In rare cases, surgery to remove the damaged section of colon may be required.

While antibiotic use is the most common precipitating cause of pseudomembranous colitis, occasionally the condition may result from **chemotherapy**, bone marrow transplantation, or other causes.

See also Microbial flora of the stomach and gastrointestinal tract

Pseudomonas

The genus *Pseudomonas* is made up of Gram-negative, rod-shaped **bacteria** that inhabit many niches. *Pseudomonas* species are common inhabitants of the soil, water, and vegetation. The genus is particularly noteworthy because of the tendency of several species to cause infections in people who are already ill, or whose immune systems are not operating properly. Such infections are termed opportunistic infections.

Pseudomonas rarely causes infections in those whose immune systems are fully functional. The disease-causing members of the genus are therefore prevalent where illness abounds. *Pseudomonas* are one of the major causes of nosocomial (hospital acquired) infections.

Bacteria in this genus not only cause infections in man, but also cause infections in plants and animals (e.g., horses). For example, *Pseudomonas mallei* causes ganders disease in horses.

The species that comprise the genus *Pseudomonas* are part of the wider family of bacteria that are classified as *Pseudomonadaceae*. There are more than 140 species in the genus. The species that are associated with opportunistic infections include *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas stutzeri*, and *Pseudomonas putrefaciens*. *Pseudomonas aeruginosa* is probably the most well-known member of the genus.

Pseudomonas are hardy **microorganisms**, and can grow on almost any available surface where enough moisture and nutrients are present. Members of the genus are prone to form the adherent bacterial populations that are termed biofilms. Moreover, *Pseudomonas aeruginosa* specifically change their genetic behavior when on a surface, such that they produce much more of the **glycocalyx** material than they produce when floating in solution. The glycocalyx-enmeshed bacteria become extremely resistant to antibacterial agents and immune responses such as **phagocytosis**.

In the hospital setting *Pseudomonas aeruginosa* can cause very serious infections in people who have cancer, cystic fibrosis, and burns. Other infections in numerous sites in the body, can be caused by *Pseudomonas spp.* Infections can be site-specific, such as in the urinary tract or the respiratory system. More widely disseminated infections (termed systemic infections) can occur, particularly in burn victims and those whose immune systems are immunosuppressed.

For those afflicted with cystic fibrosis, the long-lasting lung infection caused by *Pseudomonas aeruginosa* can ultimately prove to be fatal. The bacteria have a surface that is altered from their counterparts growing in natural environ-

ments. One such alteration is the production of a glycocalyx around the bacteria. The bacteria become very hard for the **immune system** to eradicate. The immune response eventually damages the epithelial cells of the lung. So much so, sometimes, that lung function is severely compromised or ceases.

Another bacterium, *Pseudomonas cepacia*, is also an opportunistic cause of lung infections in those afflicted with cystic fibrosis. This species is problematic because it is resistant to more **antibiotics** than is *Pseudomonas aeruginosa*.

Glycocalyx production in some strains of *Pseudomonas aeruginosa* can be so prodigious that colonies growing on solid media appear slimy. Indeed, some species produce such mucoid colonies that the colonies will drip onto the lid of the **agar** plate when the plate is turned upside down. These slimy growths are described as mucoid colonies, and are often a hallmark of a sample that has been recovered from an infection.

Disease-causing species of *Pseudomonas* can possess a myriad of factors in addition to the glycocalyx that enable a bacterium to establish an infection. The appendages known as pili function in adherence to host cells. A component of the outer membrane possesses an endotoxin. Finally, a number of exotoxins and extracellular **enzymes** can cause damage at a distance from the bacterium. One such exotoxin, which is called toxin A, is extremely potent, and may be the prime cause of damage by the bacteria in infections.

Some species, especially *Pseudomonas aeruginosa* are a problem in hospitals. By virtue of their function, hospitals are a place where many immunocompromised people are found. This is an ideal environment for an opportunistic disease-causing bacterium. Moreover, *Pseudomonas aeruginosa* has acquired resistance to a number of commonly used antibiotics. As yet, a **vaccine** to the bacterium does not exist. Prevention of the spread of *Pseudomonas* involves the observance of proper **hygiene**, including handwashing.

See also Bacteria and bacterial infection; Infection and resistance; Lipopolysaccharide and its constituents

PSYCHROPHILIC BACTERIA

Psychrophilic ("cold loving") **microorganisms**, particularly **bacteria**, have a preferential temperature for growth at less than 59° Fahrenheit (15° Celsius). Bacteria that can grow at such cold temperatures, but which prefer a high growth temperature, are known as psychrotrophs.

The discovery of psychrophilic microorganisms and the increasing understanding of their functioning has increased the awareness of the diversity of microbial life on Earth. So far, more than 100 varieties of psychrophilic bacteria have been isolated from the deep sea. This environment is very cold and tends not to fluctuate in temperature. Psychrophilic bacteria are abundant in the near-freezing waters of the Arctic and the Antarctic. Indeed, in Antarctica, bacteria have been isolated from permanently ice-covered lakes. Other environments where psychrophilic bacteria have been include high altitude cloud droplets.

Psychrophilic bacteria are truly adapted for life at cold temperatures. The **enzymes** of the bacteria are structurally unstable and fail to operate properly even at room (or ambient) temperature. Furthermore, the membranes of psychrophilic bacteria contain much more of a certain kind of lipid than is found in other types of bacteria. The lipid tends to be more pliable at lower temperature, much like margarine is more pliable than butter at refrigeration temperatures. The increased fluidity of the membrane makes possible the chemical reactions that would otherwise stop if the membrane were semi-frozen. Some psychrophiles, particularly those from the Antarctic, have been found to contain polyunsaturated fatty acids, which generally do not occur in prokaryotes. At room temperature, the membrane of such bacteria would be so fluid that the bacterium would die.

Aside from their ecological curiosity, psychrophilic bacteria have practical value. Harnessing the enzymes of these organisms allows functions such as the cleaning of clothes in cold water to be performed. Furthermore, in the Arctic and Antarctic ecosystems, the bacteria form an important part of the food chain that supports the lives of more complex creatures. In addition, some species of psychrophiles, including *Listeria monocytogenes* are capable of growth at refrigeration temperatures. Thus, spoilage of contaminated food can occur, which can lead to disease if the food is eaten. Listeriosis, a form of **meningitis** that occurs in humans, is a serious health threat, especially to those whose **immune system** is either not mature or is defective due to disease or therapeutic efforts. Other examples of such disease-causing bacteria include *Aeromonas hydrophila*, *Clostridium botulinum*, and *Yersinia enterocolitica*.

See also Extremophiles

PUBLIC HEALTH, CURRENT ISSUES

Public health is the establishment and maintenance of healthful living conditions for the general population. This goal requires organized effort from all levels of government. Underlying the current concerns in public health are three principle aims of public health efforts. First is the assessment and monitoring of populations, from the community level to the national level, to identify populations who are at risk for whatever health problem is being considered. For example, public health efforts have shown that aboriginals in Canada are especially prone to developing diabetes. The second "plank" of public health is the formulation of policies to deal with the significant problems. Returning to the example, policies and strategies for action are now being formulated to reverse the trend. The third core public health function is to assure that everyone is able to receive adequate and affordable care and disease prevention services.

There are many microbiological threats to public health. In order to maintain the three cores of public health, priorities must be established. In organizations such as the **Centers for Disease Control** and the **World Health Organization**, different divisions have been created to address the different concerns. Within each division the particular area of concern, such as

Ciprofloxacin used to treat anthrax.

food safety, can be simultaneously addressed at various levels, including basic research, policy development, and public awareness.

In the aftermath of the September 11, 2001, terrorist attacks on targets in the United States, public perception of the health risks of what is commonly known as **bioterrorism** has been heightened. The ability to transport harmful **microorganisms** or their products, such as **anthrax**, through the mail or via dispersal in the air has made clear how vulnerable populations are to attack. Public health agencies have realized that the ability to promptly respond to an incident is critical to any successful containment of the disease causing microbial threat. But the achievement of this response will require a huge effort from many public and private agencies, and will be extremely expensive. For example, it has been estimated that a response to each incident of bioterrorism, real or not, costs on the order of 50,000 dollars. Repeated mobilization of response teams would quickly sap the public health budget, at the cost of other programs. Thus, in the latter years of the twentieth century and the new century, the issue of bioterrorism and how to deal with it in a safe and economically prudent way has become a paramount public health issue.

Another public health issue that has become more important is the emergence of certain microbial diseases. In the emergence category, **hemorrhagic diseases** of viral origin, such as Ebola and Lassa fever are appearing more frequently. These diseases are terrifying due to their rapid devastation inflicted on the victim of infection, and because treatments are as yet rudimentary. The emergence of such diseases, which seems to be a consequence of man's encroachment on environments that have been largely untouched until now, is a harbinger of things to come. Public health agencies are moving swiftly to understand the nature of these diseases and how to combat them.

Diseases are also re-emerging. **Tuberculosis** is one example. Diseases such as tuberculosis were once thought to be a thing of the past, due to **antibiotics** and public health initiatives. Yet, the numbers of people afflicted with such diseases is on the rise. One factor in the re-emergence of certain diseases is the re-acquisition of **antibiotic resistance** by **bacteria**. Another factor in the re-emergence of tuberculosis is the sharp increase in the number of immunocompromised individuals that are highly susceptible to tuberculosis, such as those with acquired immune deficiency syndrome (**AIDS**). The overuse and incomplete use of antibiotics has also enabled bacteria to develop resistance that can be passed on to subsequent generations. Public health efforts and budgets are being

re-directed to issues thought at one time to be dealt with and no longer a concern.

Certain infectious diseases represent another increasingly important public health issue. Just a few decades ago AIDS was more of a curiosity, given its seeming confinement to groups of people who were often marginalized and ostracized. In the past decade, however, it has become clear that AIDS is an all-inclusive disease. Aside from the suffering that the illness inflicts, the costs of care for the increasingly debilitated and dependent patients will constitute a huge drain on health care budgets in the decades to come. As a result, AIDS research to develop an effective **vaccine** or strategies that prolong the vitality of those infected with the AIDS virus is a major public health issue and priority.

Another public health issue of current importance is chronic bacterial and viral diseases. Conditions like fibromyalgia may have a bacterial or viral cause. The chronic and debilitating **Lyme disease** certainly has a bacterial cause. Moreover, the increasing use of surgical interventions to enhance the quality of life, with the installation of heart pacemakers, artificial joints, and the use of catheters to deliver and remove fluids from patients, has created conditions conducive for the explosion in the numbers of bacterial infections that result from the colonization of the artificial surfaces. Such bacterial biofilms have now been proven to be the source of infections that persist, sometimes without symptoms, in spite of the use of antibiotics. Such infections can be life threatening, and their numbers are growing. As with the other current public health issues, chronic infections represent both a public health threat and a budget drain.

A final area that has long been a public health concern is the safety of food and water. These have always been susceptible to **contamination** by bacteria, **protozoa** and **viruses**, in particular. With the popularity of prepared foods, the monitoring of foods and their preparation has become both more urgent and more difficult for the limited number of inspectors to do. Water can easily become contaminated. The threat to water has become greater in the past twenty years, because of the increasing encroachment of civilization on natural areas, where the protozoan pathogens *Giardia* and *Cryptosporidium* normally live, and because of the appearance of more dangerous bacterial pathogens, in particular ***Escherichia coli*** O157:H7. The latter organism is a problem in food as well.

See also Bacteria and bacterial infection; Epidemics and pandemics; Food safety; History of public health; Viruses and responses to viral infection

PUBLIC HEALTH SYSTEMS • *see* HISTORY OF PUBLIC HEALTH

PULSE-CHASE EXPERIMENT • *see* LABORATORY TECHNIQUES IN IMMUNOLOGY

PYREX: CONSTRUCTION, PROPERTY, AND USES IN MICROBIOLOGY

Pyrex is a brand name of a type of glass that is constructed of borosilicate. The Corning Glass Company of Corning, New York, developed Pyrex. Chemically, as borosilicate implies, this type of glass is composed of silica and at least five percent (of the total weight of the elements in the glass) of a chemical called boric oxide. The combination and concentrations of these constituents confers great resistance to temperature change and corrosion by harsh chemicals, such as strong acids and alkalis, to whatever vessel is made of the borosilicate glass. This durability has made Pyrex glassware extremely useful in the microbiology laboratory.

The development of Pyrex in 1924 by scientists at the Corning Company satisfied the demand for high quality scientific glassware that had began in the nineteenth century. Then, the glassware in existence was degraded by laboratory chemicals and became brittle when exposed to repeated cycles of heating and cooling. The formulation of Pyrex minimized the tendency of the material to expand and contract. This maintained the accuracy of measuring instruments such as graduated cylinders, and overcame the brittleness encountered upon repeated autoclave **sterilization** of the laboratory glassware.

Pyrex glassware immediately found acceptance in the microbiology research community. The popularity of the glassware continues today, despite the development of heat and chemical resistant plastic polymers. Glass is still the preferred container for growing **bacteria**. This is because the glass can be cleaned using harsh chemicals, which will completely remove any organic material that might otherwise adhere to the sides of the vessel. For applications where the chemical composition and concentrations of the medium components are crucial, such organic contaminants must be removed.

Pyrex glassware is also used to manufacture graduated cylinders that are extremely accurate. In some applications, the exact volume of a liquid is important to achieve. This type of glassware is known as volumetric glassware. Plastic still cannot match the accuracy or the unchanging efficiency of volume delivery that is achieved by Pyrex volumetric glassware.

Another application for borosilicate glass is in the measurement of optical density. For this application, typically specially designed vials are filled with the solution or suspension of interest and then placed in the path of a beam of light in a machine known as a **spectrophotometer**. The amount of light that passes through the sample can be recorded and, with the inclusion of appropriate controls, can be used, for example, to determine the number of bacteria in the sample. Plastic material does not lend itself to optical density measurements, as the plastic can be cloudy. Thus, the vial itself would absorb some of the incoming light. Pyrex, however, can be made so as to be optically transparent. Growth flasks have even been made in which a so-called "side arm," basically a test tube that is fused onto the flask, can be used to directly obtain optical density measurements without removing the **culture** from the flask.

In the same vein, the use of optically transparent slabs of Pyrex as **microscope** slides is a fundamental tool in the micro-

Pyrex labware filled with colored liquid.

biology laboratory. The heat resistance of the slide allows a specimen to be heated directly on the slide. This is important for stains such as the acid-fast stain for mycobacteria, in which heating of the samples is essential for the accurate staining of the bacteria. Also, as for the optical density measurements, the light microscopic examination of the bacterial sample depends upon the transparency of the support surface. Plastic is not an appropriate support material for slides.

Another area in which Pyrex glassware is essential in a microbiology laboratory is in the pipelines required for the delivery of distilled water. Distillation of water is a process that requires the boiling of the water. The pipelines must be heat resistant. Also, because physical scrubbing of the pipelines is not feasible, the pipes must withstand the application of caustic chemicals to scour organic material off the interior surface of the pipes.

Other applications of borosilicate glassware in the microbiology laboratory include nondisposable Petri plates for the use of solid media, centrifuge tubes, titration cylinders, and the stopcocks that control the flow rate.

Heat and chemically resistant plastics are widely used in the typical microbiology laboratory, particularly for routine, high-volume operations where cleaning and preparation of glassware for re-use is time-consuming and prone to error. However, the accuracy and advantages of Pyrex glassware ensure its continued use in the most modern of microbiology laboratories.

See also Laboratory methods in microbiology; Microscopy

PYRROPHYTA

Approximately 2000 species of Pyrrophyta (from the Greek *pyrrhos,* meaning flames, and *phyton,* meaning plant) are known at present. Pyrrophyta have been identified in fossil deposits around the globe, from arctic to tropical seas, as well as in hypersaline waters, freshwater, and river deltas. Pyrrophyta are mostly unicellular microorganic **Protists** divided by botanists in two phyla, **dinoflagellates** and criptomonads.

The taxonomic classification of Pyrrophyta is disputed by some zoologists who consider them members of the **Protozoa** kingdom. Cryptomonads for instance, are considered red-brownish algae of Cryptomonadida Order by botanists, and protozoans of Cryptophycea Class by zoologists. This controversy is due to the unusual characteristics of these two phyla, sharing features with both plants and animals. For instance, most species swim freely because of the spiraling

agitation of two flagella, and have multiple cell walls with two valves. Some Pyrrophyta are photosynthetic species, however, whereas others are not. They come in a variety of shapes and sizes and the photosynthetic species have golden-brown or yellowish-green chloroplasts. They can synthesize both types of **chlorophyll**, type a and type c, and contain high levels of carotenoids (yellow pigments). Some Pyrrophyta, such as *Gymnodium* and *Gonyaulax* are dinoflagellates responsible for red tides and secrete neurotoxins that cause massive fish death. If these toxins are airborne in a closed room, or if they get in contact with the skin, they may contaminate humans and cause temporary or more severe neurological disorders. Some species such as the *Ceratium* can deplete water from oxygen, also leading to massive fish death, a phenomenon known as black tide.

Photosynthetic Pyrrophyta are autotrophs, whereas the non-photosynthetic ones may be heterotrophs, existing as **parasites** in fish and aquatic invertebrates as well. Some autothrophic species also feed on other dinoflagellates and unicellular organisms, by engulfing them. Symbiotic species (zooxanthellae) are also known, which live in sponges, jellyfish, anemones, growing coral reefs, etc, where they supply carbon to their hosts. Cryptomonads themselves are the evolutionary result of endosymbiosis, and are chimeric species that evolved from ancestral red algae and a non-photosynthetic host that retained the red alga **nucleus** under the form of a bead-like nucleomorph chromosome. The highly condensed chromosome of this Pyrrophyta consists of three different bead-like nucleomorphic units.

See also Chromosomes, eukaryotic; Photosynthesis

Q

Q FEVER

Q (or Query) fever is a disease that is caused by the bacterium *Coxiella burnetii*. The bacterium is passed to humans by contact with infected animals such as sheep, cattle, and goats, which are the main reservoirs of the microorganism. The disease, which was first described in Australia in 1935, can have a short-term (acute) stage and, in some people, a much longer, chronic stage.

The bacterium that causes Q fever is a **rickettsia**. Other rickettsia are responsible for Rocky Mountain Spotted Fever and trench fever, as examples. *Coxiella burnetti* and the other rickettsia are Gram-negative organisms, which need to infect host cells in order to grow and divide. Outside of the host the **bacteria** can survive, but do not replicate. Q fever differs from the other rickettsial diseases in that it is caused by the inhalation of the bacteria, not by the bite of a tick.

Groups most at risk to acquire Q fever are those who are around animals. These include veterinarians, sheep, cattle and dairy farmers, and workers in processing plants.

The bacteria are excreted into the environment in the milk, urine, and feces of the animals. Also, bacteria can be present in the amniotic fluid and the placenta in the birthing process. The latter is particularly relevant, as humans tend to be near the animals during birth, and so the chances of transfer of the bacterium from animal to human are great.

In addition, the **microorganisms** are hardy and can endure environmental stress. The chances for human infection are also increased because of the persistence of the bacteria in the environment outside of the animal host. *Coxiella burnetii* are very hardy bacteria, being resistant to antibacterial compounds, and to environmental stresses such as heat and lack of moisture. When present in a dry area, such as in hay or the dust of a barnyard, the organisms can be easily inhaled.

The entry of only a few live bacteria or even one living bacterium is required to cause an infection in humans. The environmental hardiness and low number of microbes required for an infection has made *Coxiella burnetii* a potential agent of **bioterrorism.**

Of those who become infected, only about half display symptoms. When symptoms of Q fever appear, they can include the sudden development of a high fever, severe headache, nausea, vomiting, abdominal pain, and an overall feeling of illness. **Pneumonia** and liver damage can develop in some people. Usually the symptoms pass in several months. However, the establishment of a chronic disease can occur, and is fatal in over 60 per cent of cases. The chronic form may not develop immediately after the transient disease. In fact, cases have been documented where the lapse between the initial disease and the chromic form was several decades. The chronic disease can lead to heart valve damage.

Why some people display symptoms of infection while others do not is still not resolved. Neither are the reasons why the disease is self-limiting within a short time in some people but develops into a lengthy, debilitating, and potentially lethal disease in other people.

Coxiella burnetii has two different forms, which have differing surface chemistries. These are called phase I and phase II. The phase I form is associated more with the chronic Q fever than is phase II.

Diagnosis of Q fever is most reliably obtained by the detection of antibodies to the infecting bacterium. Following diagnosis, treatment consists of antibiotic therapy. The **antibiotics** that have achieved the most success are fluoroquinolone, rifampin, and trimethoprim-sulfamethoxazole. In the chronic form of Q fever, the antibiotics may need to be administered for several years. If the disease has damaged body parts, such as heart valve, then treatment may also involve the replacement of the damaged tissues.

Vaccination against Q fever is not yet a standard option. A **vaccine** is available in Australia and parts of Europe, but has not yet been approved in North America.

Prevention of the transmission of the bacterium to humans involves the wearing of masks when around domestic

Mountain sheep, one of the natural hosts of the Q-fever bacterium *Coxiella burnetii*.

animals and the prompt disposal of placenta and other tissues resulting from the birth process.

See also Bacteria and bacterial diseases; Zoonoses

QUALITATIVE AND QUANTITATIVE ANALYSIS IN MICROBIOLOGY

Various techniques have been devised to permit the analysis of the structure and function of **microorganisms**. Some techniques are qualitative in their intent. That is, they provide a "yes or no" answer. Other techniques are quantitative in their intent. These techniques provide numerical information about a sample.

Assessing the growth of a bacterial sample provides examples of both types of analysis techniques. An example of a qualitative technique would be the growth of a bacterial sample on a solid growth medium, in order to solely assess whether the **bacteria** in the sample are living or dead. An example of a quantitative technique is the use of that solid growth media to calculate the actual number of living bacteria in a sample.

Microscopic observation of microorganisms can reveal a wealth of qualitative information. The observation of a suspension of bacteria on a **microscope** slide (the wet mount) reveals whether the bacteria are capable of self-propelled motion. Microorganisms, particularly bacteria, can be applied to a slide as a so-called smear, which is then allowed to dry on the slide. The dried bacteria can be stained to reveal, for example, whether they retain the primary stain in the Gram stain protocol (Gram positive) or whether that stain is washed out of the bacteria and a secondary stain retained (Gram negative). Examination of such smears will also reveal the shape, size, and arrangement (singly, in pairs, in chains, in clusters) of the bacteria. These qualitative attributes are important in categorizing bacteria.

Microscopy can be extended to provide qualitative information. The incorporation of antibodies to specific components of the sample can be used to calculate the proportion

Growth of bacteria on agar is a qualitative result.

of the samples in a population that possess the target of interest. Fluorescent-labeled antibodies, or antibodies combined with a dark appearing molecule such as ferritin, are useful in such studies. The scanning confocal microscope is proving to be tremendously useful in this regard. The optics of the microscope allows visual data to be obtained at various depths through a sample (typically the sample is an adherent population of microorganisms). These optical thin sections can be reconstructed via computer imaging to produce a three-dimensional image of the specimen. The use of fluorescent-tagged antibodies allows the location of protein within the living biofilm to be assessed.

The self-propelled movement of living microorganisms, a behavior that is termed motility, can also provide quantitative information. For example, recording a moving picture image of the moving cells is used to determine their speed of movement, and whether the presence of a compound acts as an attractant or a repellant to the microbes.

Bacterial growth is another area that can yield qualitative or quantitative information. Water analysis for the bacterium ***Escherichia coli*** provides an example. A specialized growth medium allows the growth of only *Escherichia coli*. Another constituent of the growth medium is utilized by the growing bacteria to produce a by-product that fluoresces when exposed to ultraviolet light. If the medium is dispensed in bottles, the presence of growing *Escherichia coli* can be detected by the development of fluorescence. However, if the medium is dispensed in smaller volumes in a grid-like pattern, then the number of areas of the grid that are positive for growth can be related to a mathematical formula to produce a most probable number of living *Escherichia coli* in the water sample. Viable bacterial counts can be determined for many other bacteria by several other means.

The ability of bacteria to grow or not to grow on a media containing controlled amounts and types of compounds yields quantitative information about the nutritional requirements of the microbes.

The advent of molecular techniques has expanded the repertoire of quantitative information that can be obtained. For example, a technique involving reporter genes can show whether a particular **gene** is active and can indicate the number of copies of the gene product that is manufactured. Gene probes have also been tagged to fluorescent or radioactive labels to provide information as to where in a population a certain metabolic activity is occurring and the course of the activity over time.

Many other qualitative and quantitative techniques exist in microbiological analysis. A few examples include **immunoelectrophoresis**, immunoelectron microscopy, biochemical dissection of metabolic pathways, the molecular construction of cell walls and other components of microorganisms, and mutational analysis. The scope of the techniques is ever-expanding.

See also Laboratory techniques in immunology; Laboratory techniques in microbiology

QUORUM SENSING

Quorum sensing is a term that refers to the coordinated behavior exhibited by a population of **bacteria**. The phenomenon involves a communication between the bacterial members of the population and, via a triggering signal, the carrying out of a particular function.

Examples of quorum sensing are the coordinated feeding behavior and the formation of spores that occur in large populations of myxobacteria and actinomycetes. Quorum sensing also occurs in bacterial biofilms, where signals between bacteria can stimulate and repress the production of the extracellular polysaccharide in different regions of the biofilm, and the exodus of portions of the population from the biofilm, in order to establish a new biofilm elsewhere.

Historically, the first indication of quorum sensing was the discovery of the chemical trigger for luminescence in the bacterium *Photobacterium fischeri* in the 1990s. At high densities of bacteria, luminescence occurs. Light production, however, does not occur at lower numbers or densities of bacteria. The phenomenon was correlated with the production of a compound whose short name is homoserine lactone. The same molecule has since been shown to trigger responses in other quorum sensing systems in other bacteria. Examples of these responses include the production of disease-causing factors by *Pseudomonas aeruginosa* and cell division in ***Escherichia coli***.

Quorum sensing enables a bacterial population to respond quickly to changing environmental conditions and, in the case of biofilms, to enable regions within the mature biofilm to perform the different functions necessary to sustain the entire community.

In *Photobacterium fischeri* the relatively **hydrophobic** ("water-hating") nature of the homoserine lactone molecule drives its diffusion into the cell wall surrounding a bacterium. Once inside the bacterium, the molecule interacts with a protein known as LuxR. The LuxR then induces the **transcription** of a region the genetic material that contains the genes that code for the luminescent proteins.

The molecular nature of the means by which quorum sensing triggers such homoserine lactone evoke a bacterial response in other bacteria is still unclear. Furthermore, the discovery of several quorum sensing systems in bacteria such as *Pseudomonas aeruginosa* indicate that multiple sensing pathways are operative, at different times or even simultaneously. For example, within a biofilm, bacteria may be actively manufacturing exopolysaccharide, repressed in the polymer's construction, growing slowly, or resuming the active growth that is the hallmark of free-floating bacteria. Resolving the molecular nature of the spectrum of quorum sensing activities could lead to strategies to disrupt the inter-cellular communication in disease processes.

See also Biofilm formation and dynamic behavior

R

Rabies

Rabies is a viral brain disease that is almost always fatal if it is not prevented with prompt treatment. The disease, which typically spreads to humans from animals through a scratch or a bite, causes **inflammation** of the brain. The disease is also called hydrophobia (meaning fear of water) because it causes painful muscle spasms in the throat that prevent swallowing. In fact, this is what leads to most fatalities in untreated cases: victims become dehydrated and die. Carriers of rabies include dogs, cats, bats, skunks, raccoons, and foxes; rodents are not likely to be infected. About 70% of rabies cases develop from wild animal bites that break the skin. Though a **vaccine** used first in 1885 is widely used, fatalities still occur due to rabies. Most fatalities take place in Africa and Asia, but some also occur in the United States. The cost of efforts to prevent rabies in the United States may be as high as $1 billion per year.

While many animal diseases cannot be passed from animal to man, rabies has long been known as an easy traveler from one species to the next. The disease was known among ancient people. The very name rabies, Latin for rage or madness, suggests the fear with which early men and women must have viewed the disease. For centuries there was no treatment, and the disease was left to run its rapid course leading to death.

Rabies is described in medical writings dating from 300 B.C., but the method of transmission or contagion was not recognized until 1804. In 1884, the French bacteriologist **Louis Pasteur** developed a preventive vaccine against rabies, and modifications of Pasteur's methods are still used in rabies therapy today. The Pasteur program, or variations of it, has greatly reduced the fatalities in humans from rabies. Modern treatment, following a bite by a rabid or presumed rabid animal, consists of immediate and thorough cleansing of the bite wound and injection into the wound and elsewhere of hyperimmune antirabies serum. Post exposure treatment consists of five injections of vaccine given over a one-month period, along with one dose of rabies immune globulin injected near the wound and intramuscularly.

The standard vaccine contains inactivated rabies virus grown in duck eggs. It is highly effective but causes neuroparalysis in about one in 30,000 persons receiving it. In the 1970s, a new vaccine was developed in France and the United States that contains virus prepared from human cells grown in the laboratory. This vaccine is safer and requires a shorter course of injections. With the widespread use of vaccine, rabies cases in the U.S. declined to fewer than five per year.

The transmission of rabies is almost invariably through the bite of an infected animal. The fact that the virus is eliminated in the saliva is of great significance, and unless saliva is introduced beneath the skin, the disease is seldom transmitted. The virus has been demonstrated in the saliva of dogs 3–8 days before the onset of symptoms. However, it has also been reported that only about 50–60% of the infected dogs shed the virus in the saliva. Rare cases of rabies have been reported where only clawing and scratching occurred, or where the skin was contaminated with saliva. The virus is most concentrated in the central nervous system and saliva, but it has also been demonstrated in various organs of the body and milk from infected animals.

In humans, the rabies virus, in addition to entering the body by the usual route through skin broken by a bite or scratch, can enter the body through intact mucous membranes, can be inhaled as an aerosol, and can be transplanted in an infected corneal graft. These four cases are the only virologically documented examples of transmission of rabies from one person to another. Vertical transmission from mother to fetus and from lactating mother to suckling young has been described in nonhuman mammals.

The incubation period in natural cases of rabies is variable. In general, the quantity of virus introduced into the wound is correlated with the length of incubation before symptoms occur. In dogs, the minimum period is ten days, the average 21–60 days, but may be as long as six months. In man, the incubation period is one to three months, with the minimum of ten days.

The raccoon is a common transmitter of the rabies virus to humans.

Rabies is caused by a number of different **viruses** that vary depending on geographic area and species. While the viruses are different, the disease they cause is singular in its course. The bullet-shaped virus is spread when it breaks through skin or has contact with a mucous membrane. The virus begins to reproduce itself initially in muscle cells near the place of first contact. At this point, within the first five days or so, treatment by **vaccination** has a high rate of success.

Once the rabies virus passes to the nervous system, **immunization** is no longer effective. The virus passes to the central nervous system, where it replicates itself in the system and moves to other tissues such as the heart, the lung, the liver, and the salivary glands. Symptoms appear when the virus reaches the spinal cord.

A bite from a rabid animal does not guarantee that one will get rabies; only about 50% of people who are bitten and do not receive treatment ever develop the disease. If one is bitten by or has had any exposure to an animal that may have rabies, medical intervention should be sought immediately. Treatment virtually ensures that one will not come down with the disease. Any delay could diminish the treatment's effectiveness.

In humans and in animals, rabies may be manifest in one of two forms: the furious (agitated) type or the paralytic (dumb) type. Furious rabies in animals, especially in the dog, is characterized by altered behavior such as restlessness, hiding, depraved appetite, excitement, unprovoked biting, aimless wandering, excessive salivation, altered voice, pharyngeal paralysis, staggering, general paralysis, and finally death. Death usually occurs within three to four days after the onset of symptoms. The paralytic form of rabies is frequently observed in animals inoculated with fixed virus, and is occasionally observed in other animals with street virus contracted under natural conditions. Animals showing this type usually show a short period of excitement followed by uncoordination, ataxia, paralysis, dehydration, loss of weight, followed by death.

In humans, furious rabies patients typically show bizarre behavior, ranging from episodes of severe agitation to periods of depression. Confusion becomes extreme as the disease progresses, and the patient may become aggressive. Hydrophobia is always seen with this type of disease, until the patient becomes comatose while showing intermittently uncontrollable inspiratory spasms. This type of rabies is also characterized by hypersalivation, from 1–1.6 qt (1–1.5 L) of saliva in 24 hours, and excessive sweating.

The paralytic form of rabies in humans is often indistinguishable from that of most viral encephalitis, except for the fact that a patient suffering from rabies remains conscious during the course of the disease. Paralysis usually begins at the extremity exposed to the bite and gradually involves other extremities finally affecting the pharyngeal and respiratory muscles.

The dog is a most important animal as a disseminator of rabies virus, not only to man but also to other animals. Wild carnivora may be infected and transmit the disease. In the United States, foxes, raccoons and skunks are the most commonly involved. These animals are sometimes responsible for infecting domestic farm animals.

The disease in wildlife (especially skunks, foxes, raccoons, and bats) has become more prevalent in recent years, accounting for approximately 85% of all reported cases of animal rabies every year since 1976. Wildlife now constitutes the most important potential source of infection for both human and domestic animals in the United States. Rabies among animals is present throughout the United States with the exception of Hawaii, which has remained consistently rabies-free. The likelihood of different animals contracting rabies varies from one place to the next. Dogs are a good example. In areas where **public health** efforts to control rabies have been aggressive, dogs make up less than 5% of rabies cases in animals. These areas include the United States, most European countries, and Canada.

However, dogs are the most common source of rabies in many countries. They make up at least 90% of reported cases of rabies in most developing countries of Africa and Asia and many parts of Latin America. In these countries, public health efforts to control rabies have not been as aggressive. Other key carriers of rabies include the fox in Europe and Canada, the jackal in Africa, and the vampire bat in Latin America.

In the United States, 60% of all rabies cases were reported in raccoons. The high number of cases in raccoons reflects an animal epidemic, or, more properly, an epizootic. The epizootic began when diseased raccoons were carried from further south to Virginia and West Virginia. Since then, rabies in raccoons has spread up the eastern seaboard of the United States. Concentrations of animals with rabies include coyotes in southern Texas, skunks in California and in south and north central states, and gray foxes in southeastern Arizona. Bats throughout the United States also develop rabies. When rabies first enters a species, large numbers of animals die. When it has been around for a long time, the species adapts, and smaller numbers of animals die.

There are few deaths from rabies in the United States. Between 1980 and the middle of 1994, a total of 19 people in the United States died of rabies, far fewer than the 200 Americans killed by lightning, for example. Eight of these cases were acquired outside the United States. Eight of the 11 cases contracted in the United States stemmed from bat-transmitted strains of rabies.

Internationally, more than 33,000 people die annually from rabies, according to the World Health Association. A great majority of cases internationally stem from dog bites. Different countries employ different strategies in the fight against rabies. The United States depends primarily on vaccination of domestic animals and on immunization following exposure to possibly rabid animals. Great Britain, in which rabies has never been established, employs a strict quarantine for all domestic animals entering the country.

Continental Europe, which has a long history of rabies, developed an aggressive program in the 1990s of airdropping a new vaccine for wild animals. The vaccine is mixed with pellets of food for red foxes, the primary carrier there. Public health officials have announced that fox rabies may be eliminated from Western Europe by the end of the decade. The **World Health Organization** is also planning to use the vaccine in parts of Africa.

Though the United States have been largely successful in controlling rabies in humans, the disease remains present in the animal population, a constant reminder of the serious threat rabies could become without successful prevention efforts.

See also Viruses and responses to viral infection

RADIATION MUTAGENESIS

Mutations are caused by **DNA** damage and genetic alterations that may occur spontaneously at a very low rate. The frequency of these mutations can be increased by using special agents called mutagens. Ionizing radiation was the first mutagen that efficiently and reproducibly induced mutations in a multicellular organism. Direct damage to the cell **nucleus** is believed to be responsible for both mutations and other radiation mediated genotoxic effects like chromosomal aberrations and lethality. Free radicals generated by irradiation of the **cytoplasm** are also believed to induce **gene** mutations even in the non-irradiated nucleus.

There are many kinds of radiations that can increase mutations. Radiation is often classified as ionizing or non-ionizing depending on whether ions are emitted in the penetrated tissues or not. X rays, gamma rays (γ), beta particle radiation (β), and alpha particle (α) radiation (also known as alpha rays) are ionizing form of radiation. On the other hand, UV radiation, like that in sunlight, is non-ionizing. Biologically, the differences between types of radiation effects fundamentally involve the way energy is distributed in irradiated cell populations and tissues. With alpha radiation, ionizations lead to an intense but more superficial and localized deposition of energy. Primary ionization in x rays or gamma radiation traverses deeper into tissues. This penetration leads to a more even distribution of energy as opposed to the more concentrated or localized alpha rays.

This principle has been used experimentally to deliver radiation to specific cellular components. A cumulative effect of radiation has been observed in animal models. This means that if a population is repeatedly exposed to radiation, a higher frequency of mutations is observed that is due to additive effect. Intensive efforts to determine the mutagenic risk of low dose exposure to ionizing radiation have been an ongoing concern because of the use of nuclear energy and especially because of the exposure to radon gas in some indoor environments. Radon is estimated by the United States Environmental Protection Agency to be the cause of more than 20,000 cases of lung cancer annually.

The relative efficiencies of the different types of radiations in producing mutations is assessed as the mutagenic effect. The mutagenic effect of radiation is generally assumed to be due to direct damage to DNA, but the identity of the specific lesions remains uncertain.

Investigation of radiation's mutagenic effects on different tissues, cells, and subcellular compartments is becoming possible by the availability of techniques and tools that allow the precise delivery of small doses of radiation and that provide better monitoring of effects. Reactive oxygen species released in irradiated cells are believed to act directly on nuclear DNA and indirectly by modifying bases that will be incorporated in DNA, or deactivating DNA repair **enzymes**. Novel microbeam alpha irradiation techniques have allowed researchers to investigate radiation-induced mutations in non-irradiated DNA. There is evidence that radiation induces changes in the cytosol that—in eukaryotes—are transmitted to the nucleus and even to neighboring cells. Direct measurement of DNA damage caused by ionizing radiation is performed by examining micronucleus formation or analysis of DNA fragments on agarose gels following treatment with specific endonucleases such as those that only cleave at certain sites. The **polymerase chain reaction** (**PCR**) is also used to detect the loss of some marker genes by large deletions. The effect of ionizing radiation on cells can also be measured by evaluating the expression level of the stress inducible p21 protein.

Critical lesions leading to mutations or killing of a cell include induction of DNA strand breaks, damaged bases, and production of abasic sites (where a single base is deleted), and—in multichromosomal organisms—large chromosomal deletions. Except for large deletions, most of these lesions can be repaired to a certain extent, and the lethal and mutagenic effect of radiation is assumed to result principally from incompletely or incorrectly repaired DNA. This view is supported by experimental studies which showed that mice given a single radiation dose, called acute dose, develop a significantly higher level of mutations than mice given the same dose of radiation over a period of weeks or months. The rapid activation of the DNA-repair pathway through p53 protein and the stress-inducible p21 protein as well as the extreme sensitivity of cells with genetic defects in DNA repair machinery support the view that the ability of the cell to repair irradiation-induced DNA damage is a limiting factor in deciding the extent of the mutagenic effects.

See also Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Immunogenetics; Molecular biology and molecular genetics; Phage genetics; Radiation resistant bacteria; Radioisotopes and their uses; Viral genetics

RADIATION-RESISTANT BACTERIA

Radiation-resistant **bacteria** encompass eight species of bacteria in a genus known as *Deinococcus*. The prototype species is *Deinococcus radiodurans*. This and the other species are capable of not only survival but of growth in the presence of radiation that is lethal to all other known forms of life.

Radiation is measured in units called rads. An instantaneous dose of 500 to 1000 rads of gamma radiation is lethal to a human. However, *Deinococcus radiodurans* is unaffected by exposure to up to 3 million rads of gamma radiation. Indeed, the bacterium, whose name translates to "strange berry that withstands radiation," holds a place in The Guinness Book of World Records as "the world's toughest bacterium."

The bacterium was first isolated in the 1950s from tins of meat that had spoiled in spite of being irradiated with a dose that was thought to be sterilizing. The classification of the bacterium as *Deinococcus radiodurans*, and the isolation, characterization, and designation of the other species has been almost exclusively due to **Robert Murray** and his colleagues at the University of Western Ontario. The various species of *Deinococcus* have been isolated from a variety of locations as disperse as elephant feces, fish, fowl, and Antarctic rocks.

The reason for the development of such radiation resistance is still speculative. But, the current consensus is that it enabled the ancient form of the bacterium to survive in regions where available water was scarce. Other organisms developed different survival strategies, one example being the ability to form the metabolically dormant spore.

Deinococcus is an ancient bacteria, believed to be some two billion years old. They may have evolved at a time when Earth was bathed in more energetic forms of radiation than now, due to a different and less screening atmosphere. One theory even suggests that the bacteria originated on another world and were brought to Earth via a meteorite.

The extraterrestrial theory is likely fanciful, however, because the bacteria are not heat resistant. Exposure to temperatures as low as 113ºF (45ºC) can be lethal to the microorganism.

There are two known reasons for the radiation resistance of species of *Deinococcus*. Firstly, the structure of the two membranes that surround the Gram-negative bacterium contributes, albeit in a minor way. By far the major reason for the radiation resistance is the bacterium's ability to rapidly and correctly repair the extensive damage caused to its genetic material by radiation.

The high energy of radioactive waves literally cut apart the double stranded molecule of **deoxyribonucleic acid** (**DNA**). These cuts occur in many places, effectively shattering the genome into many, very small fragments. *Deinococcus* is able to quickly reassemble the fragments in their correct order and then slice them back together. In contrast, bacteria such as

Escherichia coli can only tolerate one or several cuts to the DNA before the radiation damage is either lethal or causes the formation of drastic **mutations**.

The molecular nature of this repair ability is not yet clear. However, the completion of the sequencing of the genome of *Deinococcus radiodurans* in late 1999 should provide the raw material to pursue this question. The genome is unique among bacteria, being comprised of four to ten pieces of DNA and a large piece of extrachromosomal DNA that is part of a structure called a plasmid. The genome of other bacteria typically consists of a single circle of DNA (although plasmid DNA can also be present). Within the chromosome-like regions of *Deinococcus* there are many repeated stretches of DNA. In an analogy to a computer, the bacterium has designed many backup copies of its information. If some back up copies are impaired, the information can be recovered from the other DNA.

This DNA repair ability has made the genus the subject of intense scrutiny by molecular biologists interested in the process of DNA manufacture and repair. Furthermore, the radiation resistance of *Deinococcus* has made the bacteria an attractive microorganism for the remediation of radioactive waste. While this use is not currently feasible at the scale that would be required to clean up nuclear contamination, small-scale tests have proved encouraging. The bacteria still need to be engineered to cope with the myriad of organic contaminants and heavy metals that are also typically part of nuclear waste sites.

See also Bioremediation; Extremophiles

RADIOISOTOPES AND THEIR USES IN MICROBIOLOGY AND IMMUNOLOGY

Radioisotopes, containing unstable combinations of protons and neutrons, are created by neutron activation that involves the capture of a neutron by the **nucleus** of an atom. Such a capture results in an excess of neutrons (neutron rich). Proton rich radioisotopes are manufactured in cyclotrons. During radioactive decay, the nucleus of a radioisotope seeks energetic stability by emitting particles (alpha, beta or positron) and photons (including gamma rays).

The history of radioisotopes in microbiology and **immunology** dates back to their first use in medicine. Although nuclear medicine traces its clinical origins to the 1930s, the invention of the gamma scintillation camera by American engineer Hal Anger in the 1950s brought major advances in nuclear medical imaging and rapidly elevated the use of radioisotopes in medicine. For example, cancer and other rapidly dividing cells are usually sensitive to damage by radiation. Accordingly, some cancerous growths can be restricted or eliminated by radioisotope irradiation. The most common forms of external radiation therapy use gamma and x rays. During the last half of the 20th century the radioisotope cobalt-60 was a frequently used source of radiation used in such treatments. Iodine-131 and phosphorus-32 are also commonly used in radiotherapy. More radical uses of radioisotopes include the use of Boron-10 to specifically attack tumor cells. Boron-10 concentrates in tumor cells and is then subjected to neutron beams that result in highly energetic alpha particles that are lethal to the tumor tissue. More modern methods of irradiation include the production of x rays from linear accelerators.

Because they can be detected in low doses, radioisotopes can also be used in sophisticated and delicate biochemical assays or analysis. There are many common laboratory tests utilizing radioisotopes to analyze blood, urine and hormones. Radioisotopes are also finding increasing use in the labeling, identification and study of immunological cells.

The study of **microorganisms** also relies heavily on the use of radioisotopes. The identification of protein species, labeling of surface components of **bacteria**, and tracing the **transcription** and **translation** steps involved in nucleic acid and protein manufacture all utilize radioisotopes.

A radioisotope can emit three different types of radiation. The first of these is known as alpha radiation. This radiation is due to alpha particles, which have a positive charge. An example is the decay of an atom of a substance called Americium to an atom of Neptunium. The decay is possible because of the release of an alpha particle.

The second type of radiation is called beta radiation. This radiation results from the release of a beta particle. A beta particle has a negative charge. An example is the decay of a carbon atom to a nitrogen atom, with the release of a beta particle.

The final type of radiation is known as gamma radiation. This type of radiation is highly energetic.

The various types of radiations can be selected to provide information on a sample of interest. For example, to examine how quickly a protein is degraded, an isotope that decays very quickly is preferred. However, to study the adherence of bacteria to a surface, a radiolabel that persisted longer would be more advantageous.

Furthermore, various radioactive compounds are used in microbiological analyses to label different constituents of the bacterial cell. Radioactive hydrogen (i.e., tritium) can be used to produce radioactive **deoxyribonucleic acid**. The radioactive **DNA** can be detected by storing the DNA sample in contact with X-ray film. The radioactive particles that are emitted from the sample will expose the film. When the film is developed, the result is an image of the DNA. This process, which is known as autoradiography, has long been used to trace the elongation of DNA, and so determine the speed at which the DNA is replicating.

DNA can also be labeled, but in a different location within the molecule, by the use of radioactive phosphorus.

Bacterial and viral proteins can be labeled by the addition of radioactive methionine to the growth mixture. The methionine, which is an amino acid, will be incorporated into proteins that are made. Several paths can then be followed. For instance, in what is known as a pulse-chase experiment, the radioactive label is then followed by the addition of nonradioactive (or "cold") methionine. The rate at which the radioactivity disappears can be used to calculate the rate of

turnover of the particular protein. In another experimental approach, the protein constituents of bacteria or **viruses** can be separated on an electrophoretic gel. The gel is then brought into contact with X-ray film. Wherever a radioactive protein band is present in the gel, the overlaying film will be exposed. Thus, the proteins that are radioactive can be determined.

The use of radiolabeled compounds that can be utilized as nutrients by bacteria allows various metabolic pathways to be determined. For example, glucose can be radiolabeled and its fate followed by various techniques, including chromatography, autoradiography, and gel **electrophoresis**. Furthermore, a molecule such as glucose can be radiolabeled at various chemical groups within the molecule. This allows an investigator to assess whether different regions of a molecule are used preferentially.

Radiolabeling has allowed for great advances in microbiological research. A well-known example is the 1952 experiment by Hershey and Chase, which established that DNA was the reservoir of genetic information. Bacterial viruses were exposed to either radioactive sulfur or phosphorus. The sulfur radiolabeled the surface of the virus, while the phosphorus labeled the DNA. Viruses were allowed to infect bacteria and then were mechanically sheared off of the bacteria. The sheared viruses were then collected separately from the bacteria. Radioactive sulfur was found in the virus suspension and radioactive phosphorus was found in the bacteria. Furthermore, the bacteria eventually produced new virus, some of which had radioactive DNA. Thus, radiolabeling demonstrated the relationship between DNA and genetic information.

See also Laboratory methods in microbiology

Rare genotype advantage

Rare **genotype** advantage is the evolutionary theory that genotypes (e.g., the genes of a bacterium or parasite) that have been rare in the recent past should have particular advantages over common genotypes under certain conditions.

Rare genotype advantage can be best illustrated by a host-parasite interaction. Successful **parasites** are those carrying genotypes that allow them to infect the most common host genotype in a population. Thus, hosts with rare genotypes, those that do not allow for infection by the pathogen, have an advantage because they are less likely to become infected by the common-host pathogen genotypes. This advantage is transient, as the numbers of this genotype will increase along with the numbers of pathogens that infect this formerly rare host. The pattern then repeats. This idea is tightly linked to the so-called Red Queen Hypothesis first suggested in 1982 by evolutionary biologist Graham Bell (1949–) (so named after the Red Queen's famous remark to Alice in Lewis Carroll's *Through the Looking Glass:* "Now here, you see, you have to run as fast as you can to stay in the same place."). In other words, genetic variation represents an opportunity for hosts to produce offspring to which pathogens are not adapted. Then, sex, mutation, and genetic **recombination** provide a moving target for the **evolution** of virulence by pathogens. Thus, hosts continually change to stay one step ahead of their pathogens, likened to the Red Queen's quote.

This reasoning also works in favor of pathogens. An example can be derived from the use of **antibiotics** on bacterial populations. Bacterial genomes harbor genes conferring resistance to particular antibiotics. Bacterial populations tend to maintain a high level of variation of these genes, even when they seem to offer no particular advantage. The variation becomes critical, however, when the **bacteria** are first exposed to an antibiotic. Under those conditions, the high amount of variation increases the likelihood that there will be one rare genotype that will confer resistance to the new antibiotic. That genotype then offers a great advantage to those individuals. As a result, the bacteria with the rare genotype will survive and reproduce, and their genotype will become more common in future generations. Thus, the rare genotype had an advantage over the most common bacterial genotype, which was susceptible to the drug.

See also Antibiotic resistance, tests for; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses

Recombinant DNA molecules

Recombinant **deoxyribonucleic acid** (**DNA**) is genetic material from different organisms that has been chemically bonded together to form a single macromolecule. The **recombination** can involve the DNA from two eukaryotic organisms, two prokaryotic organisms, or between an eukaryote and a prokaryote. An example of the latter is the production of human insulin by the bacterium *Escherichia coli,* which has been achieved by splicing the **gene** for insulin into the *E. coli* genome such that the insulin gene is expressed and the protein product formed.

The splicing of DNA from one genome to another is done using two classes of **enzymes**. Isolation of the target DNA sequence is done using **restriction enzymes**. There are well over a hundred restriction enzymes, each cutting in a very precise way a specific base of the DNA molecule. Used singly or in combination, the enzymes allow target segments of DNA to be isolated. Insertion of the isolated DNA into the recipient genome is done using an enzyme called DNA ligase.

Typically, the recombinant DNA forms part of the DNA making up a plasmid. The mobility of the plasmid facilitates the easy transfer of the recombinant DNA from the host organism to the recipient organism.

Paul Berg of Stanford University first achieved the manufacture of recombinant DNA in 1972. Berg isolated a gene from a human cancer-causing monkey virus, and then ligated the **oncogene** into the genome of the bacterial virus lambda. For this and subsequent recombinant DNA studies (which followed a voluntary one-year moratorium from his research while safety issues were addressed) he was awarded the 1980 Nobel Prize in chemistry.

In 1973, Stanley Cohen and Herbert Boyer created the first recombinant DNA organism, by adding recombinant **plasmids** to *E. coli*. Since that time, advances in **molecular biology** techniques, in particular the development of the **polymerase chain reaction**, have made the construction of recombinant DNA swifter and easier.

Recombinant DNA has been of fundamental importance in furthering the understanding of genetic regulatory processes and shows great potential in the genetic design of therapeutic strategies.

See also Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Laboratory techniques in immunology; Laboratory techniques in microbiology; PCR; Plasmid and plastid

RECOMBINATION

Recombination, is a process during which genetic material is shuffled during reproduction to form new combinations. This mixing is important from an evolutionary standpoint because it allows the expression of different traits between generations. The process involves a physical exchange of nucleotides between duplicate strands of **deoxyribonucleic acid** (**DNA**).

There are three types of recombination; homologous recombination, specific recombination and **transposition**. Each type occurs under different circumstances. Homologous recombination occurs in **eukaryotes**, typically during the first phase of the meiotic cell division cycle. In most eukaryotic cells, genetic material is organized as **chromosomes** in the **nucleus**. A nick is made on the chromosomal DNA of corresponding strands and the broken strands cross over, or exchange, with each other. The recombinant region is extended until a whole **gene** is transferred. At this point, further recombination can occur or be stopped. Both processes require the creation of another break in the DNA strand and subsequent sealing of the nicks by special **enzymes**.

Site specific recombination typically occurs in prokaryotes. It is the mechanism by which viral genetic material is incorporated into bacterial chromosomes. The event is site-specific, as the incorporation (integration) of viral genetic material occurs at a specific location on the bacterial genome, called the attachment site, which is homologous with the phage genome. Under appropriate conditions alignment and merging of the viral and bacterial genomes occurs.

Transposition is a third type of recombination. It involves transposable elements called **transposons**. These are short segments of DNA found in both prokaryotes and eukaryotes, which contain the information enabling their movement from one genome to another, as well as genes encoding other functions. The movement of a transposon, a process of transposition, is initiated when an enzyme cuts DNA at a target site. This leaves a section that has unpaired nucleotides. Another enzyme called transposase facilitates insertion of the transposon at this site. Transposition is important in genetic engineering, as other genes can be relocated along with the transposon DNA. As well, transposition is of natural significance. For example, the rapid reshuffling of genetic information possible with transposition enables immunocytes to manufacture the millions of different antibodies required to protect eukaryotes from infection.

See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Microbial genetics

RED TIDE

Red tides are a marine phenomenon in which water is stained a red, brown, or yellowish color because of the temporary abundance of a particular species of pigmented **dinoflagellates** (these events are known as "blooms"). Also called phytoplankton, or planktonic algae, these single-celled organisms of the class Dinophyceae move using a tail-like structure called a flagellum. They also photosynthesize, and it is their photosynthetic pigments that can tint the water during blooms. Dinoflagellates are common and widespread. Under appropriate environmental conditions, various species can grow very rapidly, causing red tides. Red tides occur in all marine regions with a temperate or warmer climate.

The environmental conditions that cause red tides to develop are not yet understood. However, they are likely related to some combination of nutrient availability, nutrient ratios, and water temperature. Red tides are ancient phenomena. Scientists suspect that human activities that affect nutrient concentrations in seawater may be having an important influence on the increasingly more frequent occurrences of red tides in some areas. In particular, the levels of nitrogen, phosphorous, and other nutrients in coastal waters are increasing due to runoff from fertilizers and animal waste. Complex global changes in climate also may be affecting red tides. Water used as ballast in ocean-going ships may be introducing dinoflagellates to new waters.

Sometimes the dinoflagellates involved with red tides synthesize toxic chemicals. Genera that are commonly associated with poisonous red tides are *Alexandrium, Dinophysis,* and *Ptychodiscus*. The algal poisons can accumulate in marine organisms that feed by filtering large volumes of water, for example, shellfish such as clams, oysters, and mussels. If these shellfish are collected while they are significantly contaminated by red-tide toxins, they can poison the human beings who eat them. Marine toxins can also affect local ecosystems by poisoning animals. Some toxins, such as that from *Ptychodiscus brevis,* the organism that causes Florida red tides, are airborne and can cause throat and nose irritations.

Red tides can cause ecological damage when the algal bloom collapses. Under some conditions, so much oxygen is consumed to support the decomposition of dead algal biomass that anoxic (lack of oxygen) conditions develop. This can cause severe stress or mortality in a wide range of organisms that are intolerant of low-oxygen conditions. Some red-tide

Red tide caused by the growth of algae in the sea.

algae can also clog or irritate the gills of fish and can cause stress or mortality by this physical effect.

Saxitoxin is a natural but potent neurotoxin that is synthesized by certain species of marine dinoflagellates. Saxitoxin causes paralytic shellfish poisoning, a toxic syndrome that affects humans who consume contaminated shellfish. Other biochemicals synthesized by dinoflagellates are responsible for diarrhetic shellfish poisoning, another toxic syndrome. Some red tide dinoflagellates produce reactive forms of oxygen—superoxide, hydrogen peroxide, and hydroxyl radical—which may be responsible for toxic effects. A few other types of marine algae also produce toxic chemicals. **Diatoms** in the genus *Nitzchia* synthesize domoic acid, a chemical responsible for amnesic shellfish poisoning in humans.

Marine animals can also be poisoned by toxic chemicals synthesized during blooms. For example, in 1991, a bloom in Monterey Bay, California, of the diatom *Nitzchia occidentalis* resulted in the accumulation of domoic acid in filter-feeding **zooplankton**. These small animals were eaten by small fish, which also accumulated the toxic chemical and then poisoned fish-eating cormorants and pelicans that died in large numbers. In addition, some humans who ate shellfish contaminated by domoic acid were made ill.

In another case, a 1988 bloom of the planktonic alga *Chrysochromulina polylepis* in the Baltic Sea caused extensive mortalities of various species of seaweeds, invertebrates, and fish. A bloom in 1991 of a closely related species of alga in Norwegian waters killed large numbers of salmon that were kept in aquaculture cages. In 1996, a red tide killed 149 endangered manatees in the coastal waters of Florida.

Even large whales can be poisoned by algal toxins. In 1985, 14 humpback whales died in Cape Cod Bay, Massachusetts, during a five-week period. This unusual mortality was caused by the whales eating mackerel that were contaminated by saxitoxin synthesized during a dinoflagellate bloom. In one observed death, a whale was seen to be behaving in an apparently normal fashion, but only 90 minutes later, it had died. The symptoms of the whale deaths were typical of the mammalian neurotoxicity that is associated with saxitoxin, and fish collected in the area had large concentrations of this poisonous chemical in their bodies.

See also Photosynthetic microorganisms; Plankton and planktonic bacteria

REPLICA PLATING • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

REPRODUCTIVE IMMUNOLOGY

Pregnant women experience many physiological changes before implantation of the early embryo (blastocyst) takes place. Ovulation, copulation, and fertilization directly or indirectly induce dramatic changes in uterine physiology that resemble classical **inflammation** at the mucosal surfaces of the female reproductive tract, and it is quite likely that these changes impact the maternal **immune system** well before the blastocyst implants in the uterus. Consequently, the outcome of the immune response differs during pregnancy, when compared to outcomes in nonpregnant women. Thus, the uterus may be preconditioned to accept the blastocyst.

Blastocyst implantation is a crucial point in the process of reproduction because it is the moment of highest spontaneous embryo loss for humans. It is characterized by the invasion of trophoblastic cells in the maternal decidua, a mucosal tissue derived from the endometrium. Antigenically, the fetus and placenta have half of the **histocompatibility** genes because of the paternal origin of the conceptus. The reasons why the fetus and placenta are accepted by the maternal immune system are still largely unknown. It is, however, a harmonic equilibrium among maternal cells of the immune system. Originally, British immunologist Peter Medawar proposed three broad hypotheses to explain the paradox of maternal immunological tolerance to the fetus: (a) physical separation of mother and fetus; (b) antigenic immaturity of the fetus; and (c) immunologic inertness of the mother. At the present time, several factors have been included in the mechanisms of fetal protection: (1) general aspecific immunosuppression due to hormonal and proteic patterns of pregnancy, (2) reduced fetal immunogenicity by alteration of expression of fetal **MHC** antigens by placental trophoblast cells, (3) IgG production toward paternal lymphocyte antigens and toward maternal lymphocytes (blocking antibodies), also called trophoblast-lymphocyte cross-reactive antigens (TLX) for their cross reactivity with antigens of the trophoblast. These blocking antibodies could bind and protect fetal antigens from maternal lymphocytes, and (4) modification of the cellular mediated response driven by **cytokines**. Cytokines are produced in the feto-placental unit and have a positive activity on the development of pregnancy.

Spontaneous human fetal loss is a significant clinical problem. Studies on recurrent spontaneous abortion syndromes are dominated by suggestions of immunologic causation. This evidence includes genetic (epidemiological) analyses, anatomical, physiological, and evidence for cytokine dysregulation linked to inappropriate activation of the innate and adaptive immune systems during human pregnancy. However, it is difficult to discriminate whether abnormalities of pregnancies are causes or effects of immune dysfunction.

Autoimmunity is defined as the pathologic condition where humoral or cellular immune response is also directed against self-antigens, leading to severe and debilitating clinical conditions. Systemic autoimmune conditions such as systemic lupus erythematosus (SLE) are associated with higher risk for pregnancy loss. In the general population, about 15% of clinical pregnancies are spontaneously aborted, and about 50% of fertilized eggs fail implantation as a blastocyst. The higher rate of fetal loss in women with SLE occurs in association with antiphospholipid antibodies (aPL), which are also associated with miscarriage in otherwise healthy women. Clinical relevance is also given to lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), and antinuclear antibodies (ANA). These are associated with several medical conditions the description of which is beyond the aim of this article.

Association of LAC with recurrent miscarriage has been described in the past twenty years. The lupus anticoagulant test (LAC) is a clotting time test used to detect women's antibodies against components of the blood clotting system, such as negatively charged **phospholipids** or prothrombin. These antibodies cause a prolongation in the clotting time.The aCL test measures 3 different species of antibodies to the phospholipid cardiolipin. This test is essentially an antiphospholipid **antibody** test, with all features similar to those of the aPL. ANA are antibodies against one or more elements within a biological cell, involved in the machinery of translating genomic message into proteins. These antibodies can destroy cells, and their effect usually leads to SLE.

When the immune system is the cause of miscarriage, the mother has a 30% chance of having a successful pregnancy without intervention after three miscarriages, a 25% chance after four miscarriages, and a 5% chance after five miscarriages. More epidemiological studies report a 90% chance of failure in untreated patients, whereas, in the presence of aPL, a 70% chance of reproductive failure was reported. Prevalence of LA in women with recurrent miscarriage has been quoted in a range between five and fifteen percent of fetal loss. Pathogenesis of fetal loss in the presence of aPL includes the presence of extensive infarction and necrosis in the placenta due the recurrent thrombosis of the placental vascular bed. In particular, intraluminal thromboses of the uterine spiral arteries and necrotizing decidual vasculopathy, histologically characterized by fibrinoid necrosis, atherosis, and intimal thickening have been observed.

Among immune system causes of miscarriage are the inability to properly detect fetal antigens and the lack of producing blocking antibodies. Another cause is maternal production of anti-sperm antibodies (IgG and IgA).

Endometriosis is a disease in which abnormal endometrial tissue grows in the abdomen and other places in the body. It causes internal bleeding, inflammation, scarring, severe pain, fatigue, and sometimes infertility. Endometriosis is related to the functional deficit of NK cells and cytoplasmic granules of cytotoxic lymphocytes (CTL) that allow the development of autoantibodies. In premature ovarian failure, autoantibodies against ovarian tissue and against gonadothropin receptors have been found. Oocyte reduction has been detected in women affected with premature ovarian failure.

Several male factors can influence the ability of successful fertilization, including the presence of male anti-sperm antibodies (IgG and IgM) that bind to the surface of the spermatozoa and may mask receptors or other functionally important proteins, thus interfering with the sperm-egg interaction, and reducing the probability for successful fertilization. Male anti-sperm antibody production is more likely to occur after vasectomy, or with undescended testicles, or epididymitis.

See also Autoimmunity and autoimmune diseases; Immunochemistry; Immunologic therapies; Immunological analysis techniques

RESPIRATION

Respiration is the physiological process that produces high-energy molecules such as adenosine triphosphate (ATP). The high-energy compounds become the fuel for the various manufacturing and growth processes of the cell. Respiration involves the transfer of electrons in a chemically linked series of reactions. The final electron acceptor in the respiration process is oxygen.

Respiration occurs in all types of organisms, including **bacteria**, **protists**, **fungi**, plants, and animals. In **eukaryotes**, respiration is often separated into three separate components. The first is known as external respiration, and is the exchange of oxygen and carbon dioxide between the environment and the organism (i.e., breathing). The second component of respiration is internal respiration. This is the exchange of oxygen and carbon dioxide between the internal body fluids, such as blood, and individual cells. Thirdly, there is cellular respiration, which is the biochemical oxidation of glucose and consequent synthesis of ATP.

Cellular respiration in prokaryotes and eukaryotes is similar. Cellular respiration is an intracellular process in which glucose is oxidized and the energy is used to make the high-energy ATP compound. ATP in turn drives energy-requiring processes such as biosynthesis, transport, growth, and movement.

In prokaryotes and eukaryotes, cellular respiration occurs in three sequential series of reactions; glycolysis, the citric acid cycle, and the electron transport chain. In prokaryotes such as bacteria, respiration involves components that are located in the **cytoplasm** of the cell as well as being membrane-bound.

Glycolysis is the controlled breakdown of sugar (predominantly, glucose, a 6-carbon carbohydrate) into pyruvate, a 3-carbon carbohydrate. Organisms frequently store complex carbohydrates, such as glycogen or starch, and break these down into glucose that can then enter into glycolysis. The process involves the controlled breakdown of the 6-carbon glucose into two molecules of the 3-carbon pyruvate. At least 10 **enzymes** are involved in glucose degradation. The oxidation of glucose is controlled so that the energy in this molecule can be used to manufacture other high-energy compounds. Each round of glycolysis generates only a small amount of ATP, in a process known as substrate-level phosphorylation. For each glucose molecule that is broken down by glycolysis, there is a net gain of two molecules of ATP. Glycolysis produces reduced nicotinamide adenine dinucleotide (NADH), a high-energy molecule that can subsequently used to make ATP in the electron transfer chain. For each glucose molecule that is broken down by glycolysis, there is a net gain of two molecules of NADH. Finally, glycolysis produces compounds that can be used to manufacture compounds that are called fatty acids. Fatty acids are the major constituents of lipids, and are important energy storage molecules.

Each pyruvate molecule is oxidized to form carbon dioxide (a 1-carbon molecule) and acetyl CoA (a two carbon molecule). Cells can also make acetyl CoA from fats and amino acids. Indeed, this is how cells often derive energy, in the form of ATP, from molecules other than glucose or complex carbohydrates. Acetyl CoA enters into a series of nine sequential enzyme-catalyzed reactions, known as the citric acid cycle. These reactions are so named because the first reaction makes one molecule of citric acid (a 6-carbon molecule) from one molecule of acetyl CoA (a 2-carbon molecule) and one molecule of oxaloacetic acid (a 4-carbon molecule). A complete round of the citric acid cycle expels two molecules of carbon dioxide and regenerates one molecule of oxaloacetic acid.

The citric acid cycle produces two high-energy compounds, NADH and reduced flavin adenine dinucleotide ($FADH_2$), that are used to make ATP in the electron transfer chain. One glucose molecule produces 6 molecules of NADH and 2 molecules of $FADH_2$. The citric acid cycle also produces guanosine triphosphate (GTP; a high-energy molecule that can be easily used by cells to make ATP) by a process known as substrate-level phosphorylation. Finally, some of the intermediates of the citric acid cycle reactions are used to make other important compounds, in particular amino acids (the building blocks of proteins), and nucleotides (the building blocks of **DNA**).

The electron transfer chain is the final series of biochemical reactions in respiration. The series of organic electron carriers are localized inside the mitochondrial membrane of eukaryotes and the single membrane of Gram-positive bacteria or the inner membrane of Gram-negative bacteria. Cytochromes are among the most important of these electron carriers. Like hemoglobin, cytochromes are colored proteins, which contain iron in a nitrogen-containing heme group. The final electron acceptor of the electron transfer chain is oxygen, which produces water as a final product of cellular respiration.

The main function of the electron transfer chain is the synthesis of 32 molecules of ATP from the controlled oxidation of the eight molecules of NADH and two molecules of $FADH_2$, made by the oxidation of one molecule of glucose in glycolysis and the citric acid cycle. The electron transfer chain slowly extracts the energy from NADH and $FADH_2$ by passing electrons from these high-energy molecules from one electron carrier to another, as if along a chain. As this occurs, protons (H+) are pumped across the membrane, creating a proton gradient that is subsequently used to make ATP by a process known as chemiosmosis.

Respiration is often referred to as aerobic respiration, because the electron transfer chain utilizes oxygen as the final electron acceptor. When oxygen is absent or in short supply,

cells may rely upon glycolysis alone for their supply of ATP. Glycolysis presumably originated in primitive cells early in the Earth's history when very little oxygen was present in the atmosphere. The glycolysis process has been referred to as anaerobic respiration, although this term is little used today to avoid confusion.

See also Bacterial growth and division; Biochemistry

RESTRICTION ENZYMES

Restriction **enzymes** are proteins that are produced by **bacteria** as a defense mechanism against **viruses** that infect the bacteria (bacterial phages). Most bacteria have restriction modification systems that consist of methylases and restriction enzymes. In such systems a bacteria's own **DNA** is modified by methylation (the addition of a methyl group, CH_3) at a specific location determined by a specific pattern of nucleotide residue and protected from degradation by specialized enzymes termed endonucleases.

The names of restriction enzymes are created from the first letter of the bacterial genus followed by the first two letters of the species plus a Roman numeral if more than one restriction enzyme has been identified in a particular species. Thus, the fifth restriction enzyme from *E. coli* is called EcoRV (pronounced e, ko, r five). Besides **cloning**, restriction enzymes are used in **genetic mapping** techniques, linking the genome directly to a conventional genetic marker.

Any DNA molecule, from viruses to humans, contains restriction-enzyme target sites purely by chance and, therefore, may be cut into defined fragments of size suitable for cloning. Restriction sites are not relevant to the function of the organism, nor would they be cut *in vivo,* because most organisms do not have restriction enzymes.

There are three types of restriction endonucleases in bacteria. Type I cuts unmodified DNA at a non-specific site 1000 base pairs beyond the recognition site. Type III recognizes a short asymmetric sequence and cuts at a site 24-26 base pairs from the recognition site. Type II recognizes short DNA of four to eight nucleotides. Type II restriction enzymes are widely used in **molecular biology**. Type II restriction enzymes have two properties useful in recombinant DNA technology. First, they cut DNA into fragments of a size suitable for cloning. Second, many restriction enzymes make staggered cuts generating single-stranded ends conducive to the formation of recombinant DNA. Hamilton Smith identified the first type II restriction enzyme, HindII, in 1970 at Johns Hopkins University.

Most type II restriction endonucleases cut DNA into staggered ends. For example, restriction enzyme EcoRI (from the bacterium ***Escherichia coli***) recognizes the following six-nucleotide-pair sequence in the DNA of any organism: 5'–GAATTC–3', 3'–CTTAAG–5'. This type of segment is called a DNA palindrome, which means that both strands have the same nucleotide sequence but in antiparallel orientation. EcoRI cuts in the six-base-pair DNA between the G and the A nucleotides. This staggered cut leaves a pair of identical single stranded ends. Some enzymes cut DNA at the same position of both strands, leaving both ends blunt.

Restriction enzymes ready for use.

See also Cell cycle (prokaryotic), genetic regulation of; DNA (Deoxyribonucleic acid); Gene amplification; Gene; Genetic code; Genetic identification of microorganisms; Genetic mapping; Genetic regulation of eukaryotic cells; Molecular biology and molecular genetics

RETROPOSONS AND TRANSPOSABLE ELEMENTS

Transposable elements are relatively long **DNA** sequences in prokaryotic and eukaryotic genomes that act as mobile genetic elements. These elements, which represent a large part of the genomes of many species transpose by a mechanism that involves DNA synthesis followed by random integration at a new target site in the genome.

All transposable elements encode for transposase, the special enzyme activity that helps in the insertion of **trans-**

posons at a new site, and most of them contain inverted repeats at their ends. The major difference between bacterial transposable elements and their eukaryotic counterparts is the mechanism of **transposition**. Only eukaryotic genomes contain a special type of transposable elements, called retroposons, which use reverse transcriptase to transpose through an **RNA** intermediate.

Transposition may result in splicing of DNA fragments into or out of the genome. During replicative transposition, the transposon is first replicated giving a new copy that is transferred to a new site, with the old copy being left at the original site. Nonreplicative transposition however describes the movement of a transposon that is excised from a donor site, usually generating a double, and is integrated in a new site.

The most basic transposable elements in **bacteria** are **insertion sequences**, which encode only for one enzyme, the transposase. Longer bacterial transposons contain at least one more protein-coding **gene**, which most frequently is an **antibiotic resistance** gene. In **eukaryotes**, retroposons are more common than transposons. They are either retroviral or nonviral. Viral retroposons encode for the **enzymes** reverse transcriptase and integrase and are flanked by long terminal repeats (LTRs) in the same way as **retroviruses**. The typical and most abundant nonviral retroposons are the short interspersed elements (SINEs) and the long interspersed elements (LINEs), which are usually repeated, many times in the mammalian genome. Both SINEs and LINEs lack LTRs and are thought to transpose through a special retrotransposition mechanism that involves **transcription** of one strand of the retroposon into RNA. This RNA undergoes conformation change (looping) and provides a primer for the synthesis of single stranded cDNA. The cDNA later serve as template for the synthesis of a double stranded DNA that is inserted in the genome by yet unknown mechanisms.

Transposons and retroposons seem to play a role in **evolution** and biology by promoting rearrangement and restructuring of genomes. Transposition may directly cause both deletion and inversion mutagenesis. Furthermore, transposable elements mediate the movement of host DNA sequences to new locations, enrich the genome with identical sequences positioned at different locations, and promote homologous **recombination**. Such recombination may eventually result in deletions, inversions, and translocations.

Transposons usually influence the expression of the genes in proximity of their insertion sites. They have therefore been extensively used as tools to create random insertion **mutants** in bacteria, **yeast** and higher eukaryotes. They are also used in large-scale functional genomic studies. They are valuable both during the **cloning** of genes and the generation of transgenic animals.

See also Microbial genetics; Transposition

RETROVIRUSES

Retroviruses are **viruses** in which the genetic material consists of **ribonucleic acid** (**RNA**) instead of the usual **deoxyribonucleic acid** (**DNA**). Retroviruses produce an enzyme known as reverse transcriptase that can transform RNA into DNA, which can then be permanently integrated into the DNA of the infected host cells.

Many **gene** therapy treatments and experiments use disabled mouse retroviruses as a carrier (vector) to inject new genes into the host DNA. Retroviruses are rendered safe by adding, mutating, or deleting viral genes so that the virus cannot reproduce after acting as a vector for the intended delivery of new genes. Although viruses are not normally affected by **antibiotics**, genes can be added to retroviruses that make them susceptible to specific antibiotics.

As of 2002, researchers have discovered only a handful of retroviruses that infect humans. **Human immunodeficiency virus** (**HIV**), the virus that causes acquired immune deficiency syndrome (**AIDS**), is a retrovirus. Another human retrovirus, **human T-cell leukemia virus** (**HTLV**), was discovered three years prior to the discovery of HIV. Both HTLV and HIV attack human immune cells called **T cells**. T cells are the linchpin of the human immune response. When T cells are infected by these retroviruses, the **immune system** is disabled and several serious illnesses result. HTLV causes a fatal form of cancer called adult T cell leukemia. HTLV infection of T cells changes the way the T cells work in the body, causing cancer. HIV infection of T cells, however, eventually kills T cells, rendering the immune system powerless to stave off infections from **microorganisms**.

Retroviruses are sphere-shaped viruses that contain a single strand or a couple of strands of RNA. The sphere-shaped capsule of the virus consists of various proteins. The capsule is studded on the outside with proteins called receptor proteins. In HIV, these receptor proteins bind to special proteins on T cells called CD4 receptors. CD4 stands for cluster of differentiation, and CD type 4 is found on specific T cells called helper cells. The human retroviruses discovered so far bind only to CD4 receptors, which makes their affinity for T helper cells highly specific.

The retrovirus receptor docks with a CD4 receptor on a T cell, and enters the T cell through the T cell membrane. Once inside, the retrovirus begins to replicate. But because the retrovirus's genetic material consists of RNA, not DNA, replication is more complicated in a retrovirus than it is for a virus that contains DNA.

In all living things, DNA is the template by which RNA is transcribed. DNA is a double-stranded molecule that is located within the **nucleus** of cells. Within the nucleus, DNA transcribes RNA, a single-stranded nucleic acid. The RNA leaves the nucleus through tiny pores and enters the **cytoplasm**, where it directs the synthesis of proteins. This process has been called the "central dogma" of genetic **transcription**. No life form has been found that violates this central dogma—except retroviruses. In retroviruses, the RNA is used to transcribe DNA, which is exactly opposite to the way genetic material is transcribed in all other living things. This reversal is why they are named retrograde, or backwards, viruses.

In addition to RNA, retroviruses contain an enzyme called reverse transcriptase. This is the enzyme that allows the retrovirus to make a DNA copy from RNA. Once this DNA

copy is made, the DNA inserts itself into the T cell's DNA. The inserted DNA then begins to produce large numbers of viral RNA that are identical to the infecting virus's RNA. This new RNA is then transcribed into the proteins that make up the infecting retrovirus. In effect, the T cell is transformed into a factory that produces more retroviruses. Because reverse transcriptase enzyme is unique to retroviruses, drugs that inhibit the action of this enzyme are used to treat retroviral infection, such as HIV. Reverse transcriptase is vital for retrovirus replication, but not for human cell replication. Therefore, modern reverse transcriptase inhibitor drugs are specific for retroviruses. Often, reverse transcriptase inhibitors are used in combination with other drugs to treat HIV infection.

Retroviruses are especially lethal to humans because they cause a permanent change in the T cell's DNA. Other viruses merely commandeer their host cell's cytoplasm and chemical resources to make more viruses; unlike retroviruses, they do not insert their DNA into the host cell's DNA. Nor do most viruses attack the body's T cells. Most people's cells, therefore, can recover from an attack from a virus. Eventually, the body's immune system discovers the infection and neutralizes the viruses that have been produced. Any cells that contain viruses are not permanently changed by the viral infection. Because retroviruses affect a permanent change within important cells of the immune system, cellular recovery from a retrovirus infection does not occur.

In 1980, researchers headed by Robert Gallo at the National Cancer Institute discovered the first human retrovirus. They found the virus within leukemic T cells of patients with an aggressive form of T cell cancer. These patients were from the southern United States, Japan, and the Caribbean. Almost all patients with this form of cancer were found to have antibodies (immune system proteins made in response to an infection) to HTLV.

HIV is perhaps the most famous retrovirus. Discovered independently by several researchers in 1983, HIV is now known to be the causative agent of AIDS. People with AIDS test positive for HIV antibodies, and the virus itself has been isolated from people with the disease.

HIV attacks T cells by docking with the CD4 receptor on its surface. Once inside the cell, HIV begins to transcribe its RNA into DNA, and the DNA is inserted into the T cell's DNA. However, new HIV is not released from the T cell right away. Instead, the virus stays latent within the cell, sometimes for 10 years or more. For reasons that are not yet clear, at some point the virus again becomes active within the T cell, and HIV particles are made within the cell. The new HIV particles bud out from the cell membrane and attack other T cells. Soon, all of the T cells of the body are infected and die. This infection cycle explains why very few virus particles are found in people with the HIV infection (those who do not yet have AIDS); many particles are found in people who have fulminate AIDS.

No cure has yet been found for AIDS. Researchers are still unsure about many aspects of HIV infection, and research into the immune system is still a relatively new science. Several anti-retroviral drugs, such as AZT, ddI, and ddC, have been administered to people with AIDS. These drugs do not cure HIV infection; but they usually postpone the development of AIDS. AIDS is almost invariably fatal.

Simian **immunodeficiency** virus (SIV) is the primate version of HIV. In fact, monkeys infected with SIV are used to test AIDS drugs for humans. Rous sarcoma virus (RSV) causes cancer in chickens and was the first retrovirus identified. Feline leukemia virus (FELV) causes feline leukemia in cats and is characterized by symptoms similar to AIDS. Feline leukemia is a serious disease that, like AIDS, is fatal. Unlike AIDS, a **vaccine** has been developed to prevent this disease.

See also AIDS, recent advances in research and treatment; Immunogenetics; T cells or T lymphocytes; Viral genetics; Viral vectors in gene therapy; Virus replication; Viruses and responses to viral infection

REVERSE TRANSCRIPTION • *see* TRANSCRIPTION

RH AND RH INCOMPATIBILITY

Human red blood cells contain protein molecules (antigens) in their cell membranes that determine the blood type of an individual. There are several kinds of antigens present on human red blood cells, as well as the Rh **antigen**. People with the Rh antigen are distinguished with a blood type ending in a plus (+); those without the Rh antigen have a minus (–) in their blood type.

Rh disease occurs when an Rh-negative mother is exposed to Rh-positive fetal blood and develops antibodies. During pregnancy, and especially during labor and delivery, some of the fetus's Rh-positive red blood cells get into the mother's (Rh -) bloodstream. Higher passage of fetal cells is observed in women who have undergone amniocentesis and other invasive diagnostic procedures, and in women with placental anomalies. This triggering of the mother's immune response is referred to as sensitization, or isoimmunization. In pregnancies occurring after exposure (usually not in the first pregnancy), maternal antibodies may lyse (disintegrate) the red blood cells of an Rh-positive fetus, leading to red blood cell destruction and fetal anemia. In the case of Rh, the predominant maternal **antibody** belongs to the G type (igG) which can freely cross the placenta and enter the fetal circulation. The consequent anemia may be so profound that the fetus may die in the uterus. Reacting to the anemia, the fetal bone marrow may release immature red blood cells (erythroblasts) into the fetal peripheral circulation, causing erythroblastosis fetalis. After birth, affected newborns may develop kernicterus. At any further pregnancy, the Rh incompatibility mechanism tends to be accelerated.

Since 1968, there has been a treatment that can prevent Rh disease. Without prophylaxis (preventative treatment), about one in six Rh negative women who deliver a Rh positive infant will develop anti-Rh antibodies from fetomaternal hemorrhage occurring either during pregnancy or at delivery. No universal policy exists for postnatal prophylaxis. The standard

dose of anti-D immunoglobulin varies in different countries. In the USA, it is standard practice for Rh– patients who deliver Rh+ infants to receive an intramuscular dose of Rh immune globulin within 72 hours after delivery. With this treatment, the risk of subsequent sensitization deceases from about 15% to 2%. However, in spite of the routine use of gammaglobulin for both antepartum and postpartum immunoprophylaxis, severe fetal Rh alloimmunization continues to be a serious medical problem. In the presence of severe fetal anemia, early intervention appears to offer substantial improvement in clinical outcome.

Prenatal antibody screening is recommended for all pregnant women at their first prenatal visit. Repeat antibody screening at 24–28 weeks gestation is recommended for unsensitized Rh-negative mothers. The goals of antepartum care are to accurately screen the pregnant woman for Rh incompatibility and sensitization, to start appropriate therapeutic interventions as quickly as possible, and to deliver a mature fetus who has not yet developed severe hemolysis.

Frequent blood tests (indirect Coombs' tests) are obtained from the mother, starting at 16 to 20 weeks' gestation. These tests identify the presence of Rh-positive antibodies in maternal blood. When the antibody titer rises to 1:16 or greater, the fetus should be monitored by amniocentesis, cordocentesis, or the delta optical density 450 test. Administration of a dose of Rh immune globulin to Rh– patients at 28 weeks was found to reduce the risk of sensitization to about 0.2%.

The early diagnosis of fetal Rh status represents the best approach for the management of the disease, and a promising non-invasive detection of incompatibility seems now possible by means of the **polymerase chain reaction** (**PCR**) analysis of cell-free fetal **DNA** circulating in the mother's blood.

See also Antibody and antigen; Antibody formation and kinetics

Rhizobium-Agrobacterium group

see Economic uses and benefits of microorganisms

Rhodophyta

The red algae phylum Rhodophyta synthesizes a class of water-soluble pigments termed phycobilins, known to be produced only by another algae, the Cryptomonads. There are approximately 6,000 species of Rhodophyta. Some of them are unicellular species that grow as filaments or membrane-like sheet cells, and some multicellular coralline species deposit calcium carbonate inside and around their cell walls, which are very similar in appearance to pink and red corals. Some Rhodophyta have an important role in coral-reef formation in tropical seas due to the deposits of calcium carbonate crystals they release in the environment, and are therefore termed coralline algae.

Rhodophyta are ancient algae whose fossil remains are found under the form of coralline algal skeletons in limestone deposits of coral reef origin dating back to the Precambrian Era. They use the blue spectrum of visible light to accomplish **photosynthesis** that allows them to live in deep waters, storing energy under the form of Floridean starch. They make mostly chlorophyll-a, and the pigments alpha and beta-carotene, phycoerythrin, as well as others similar to those made by Cyanobacteria, such as allophycocyanin and r-phycocyanin. The cell walls are made mainly of cellulose (but some species use xylan), and colloidal substances, such as agars and carageenan; and the cells may be multinucleated. The Floridean starch, a carbohydrate molecule consisting of 15 units of glucose, is kept free in the **cytoplasm**, whereas in other algae it is attached to the **chloroplast**. Some species are consumed by humans such as the Japanese nori (*Porphyra*) and others are utilized as components in processed food and by the pharmaceutical industries, such as *Chondrus,* and *Gelidium.*

See also Blue-green algae; Petroleum microbiology; Protists; Xanthophylls

Ribonucleic acid (RNA)

Nucleic acids are complex molecules that contain a cell's genetic information and the instructions for carrying out cellular processes. In eukaryotic cells, the two nucleic acids, ribonucleic acid (RNA) and **deoxyribonucleic acid** (**DNA**), work together to direct **protein synthesis**. Although it is DNA that contains the instructions for directing the synthesis of specific structural and enzymatic proteins, several types of RNA actually carry out the processes required to produce these proteins. These include messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). Further processing of the various RNA's is carried out by another type of RNA called small nuclear RNA (snRNA). The structure of RNA is very similar to that of DNA, however, instead of the base thymine, RNA contains the base uracil. In addition, the pentose sugar ribose is missing an oxygen atom at position two in DNA, hence the name deoxy-.

Nucleic acids are long chain molecules that link together individual nucleotides that are composed of a pentose sugar, a nitrogenous base, and one or more phosphate groups.

The nucleotides, the building blocks of nucleic acids, in ribonucleic acid are adenylic acid, cytidylic acid, guanylic acid, and uridylic acid. Each of the RNA subunit nucleotides carries a nitrogenous base: adenylic acid contains adenine (A), cytidylic acid contains cytosine (C), guanylic acid contains guanine (G), and uridylic acid contains uracil.

In humans, the DNA molecule is made of phosphate-base-sugar nucleotide chains, and its three-dimensional shape affects its genetic function. In humans and other higher organisms, DNA is shaped in a two-stranded spiral helix organized into structures called **chromosomes**. In contrast, most RNA molecules are single-stranded and take various shapes.

Nucleic acids were first identified by the Swiss biochemist Johann Miescher (1844–1895). Miescher isolated a cellular substance containing nitrogen and phosphorus.

Thinking it was a phosphorus-rich nuclear protein, Miescher named it nuclein.

The substance identified by Miescher was actually a protein plus nucleic acid, as the German biochemist Albrecht Kossel discovered in the 1880s. Kossel also isolated nucleic acids' two purines (adenine and guanine) and three pyrimidines (thymine, cytosine, and uracil), as well as carbohydrates.

The American biochemist Phoebus Levene, who had once studied with Kossel, identified two nucleic acid sugars. Levene identified ribose in 1909 and deoxyribose (a molecule with less oxygen than ribose) in 1929. Levene also defined a nucleic acid's main unit as a phosphate-base-sugar nucleotide. The nucleotides' exact connection into a linear polymer chain was discovered in the 1940s by the British organic chemist Alexander Todd.

In 1951, American molecular biologist James Watson and the British molecular biologists **Francis Crick** and Maurice Wilkins developed a model of DNA that proposed its now accepted two-stranded helical shape in which adenine is always paired with thymine and guanine is always paired with the cytosine. In RNA, uracil replaces thymine.

During the 1960s, scientists discovered that three consecutive DNA or RNA bases (a codon) comprise the **genetic code** or instruction for production of a protein. A **gene** is transcribed into messenger RNA (mRNA), which moves from the **nucleus** to structures in the **cytoplasm** called **ribosomes**. Codons on the mRNA order the insertion of a specific amino acid into the chain of amino acids that are part of every protein. Codons can also order the **translation** process to stop. Transfer RNA (tRNA) molecules already in the cytoplasm read the codon instructions and bring the required amino acids to a ribosome for assembly.

Some proteins carry out cell functions while others control the operation of other genes. Until the 1970s cellular RNA was thought to be only a passive carrier of DNA instructions. It is now known to perform several enzymatic functions within cells, including transcribing DNA into messenger RNA and making protein. In certain **viruses** called **retroviruses**, RNA itself is the genetic information. This, and the increasing knowledge of RNA's dynamic role in DNA cells, has led some scientists to argue that RNA was the basis for Earth's earliest life forms, an environment termed the RNA World.

The first step in protein synthesis is the **transcription** of DNA into mRNA. The mRNA exits the nuclear membrane through special pores and enters the cytoplasm. It then delivers its coded message to tiny protein factories called ribosomes that consist of two unequal sized subunits. Some of these ribosomes are found floating free in the cytosol, but most of them are located on a structure called rough endoplasmic reticulum (rER). It is thought that the free-floating ribosomes manufacture proteins for use within the cell (cell proliferation), while those found on the rER produce proteins for export out of the cell or those that are associated with the cell membrane.

Genes transcribe their encoded sequences as a RNA template that plays the role of precursor for messenger RNA (mRNA), being thus termed pre-mRNA. Messenger RNA is formed through the splicing of exons from pre-mRNA into a sequence of codons, ready for protein translation. Therefore, mRNA is also termed mature mRNA, because it can be transported to the cytoplasm, where protein translation will take place in the ribosomal complex.

Transcription occurs in the nucleus, through the following sequence of the events. The process of gene transcription into mRNA in the nucleus begins with the original DNA nitrogenous base sequence represented in the direction of transcription (e.g. from the 5' [five prime] end to the 3' [three prime] end) as DNA 5'...AGG TCC TAG TAA...3' to the formation of pre-mRNA (for the exemplar DNA cited) with a sequence of 3'...TCC AGG ATC ATT...5' (exons transcribed to pre-mRNA template) then into a mRNA sequence of 5'...AGG UCC UAG UAA...3' (codons spliced into mature mRNA).

Messenger RNA is first synthesized by genes as nuclear heterogeneous RNA (hnRNA), being so called because hnRNAs varies enormously in their molecular weight as well as in their nucleotide sequences and lengths, which reflects the different proteins they are destined to code for translation. Most hnRNAs of eukaryotic cells are very big, up to 50,000 nucleotides, and display a poly-A tail that confers stability to the molecule. These molecules have a brief existance, being processed during transcription into pre-mRNA and then in mRNA through splicing.

The molecular weight of mRNAs also varies in accordance with the protein size they encode for during translation. Because three nucleotides are needed for the translation of each amino acid that will constitute the polypeptide chain during protein synthesis, they necessarily are much bigger than the protein itself. Prokaryotic mRNA molecules usually have a short existence of about 2–3 minutes, but the fast bacterial mRNA turnover allows for a quick response to environmental changes by these unicellular organisms. In mammals, the average life span of mRNA goes from 10 minutes up to two days. Therefore, eukaryotic cells in mammals have different molecules of mRNA that show a wide range of different degradation rates. For instance, mRNA of regulatory proteins, involved either in cell **metabolism** or in the **cell cycle** control, generally has a short life of a few minutes, whereas mRNA for globin has a half-life of 10 hours.

The enzyme RNA-polymerase II is the transcriptional element in human eukaryotic cells that synthesizes messenger RNA. The general chemical structure of most eukaryotic mRNA molecules contain a 7-methylguanosine group linked through a triphosphate to the 5' extremity, forming a cap. At the other end (i.e., 3' end), there is usually a tail of up to 150 adenylils or poly-A. One exception is the histone mRNA that does not have a poly-A tail. It was also observed the existence of a correlation between the length of the poly-A tail and the half-life of a given mRNA molecule.

At the biochemical level, RNA molecules are linear polymers that share a common basic structure comprised of a backbone formed by an alternating polymer of phosphate groups and ribose (a sugar containing five carbon atoms). Organic nitrogenous bases i.e., the purines adenine and guanine, and the pyrimidines cytosine and uracil are linked together through phosphodiester bridges. These four nitrogenous bases are also termed heterocyclic bases and each of

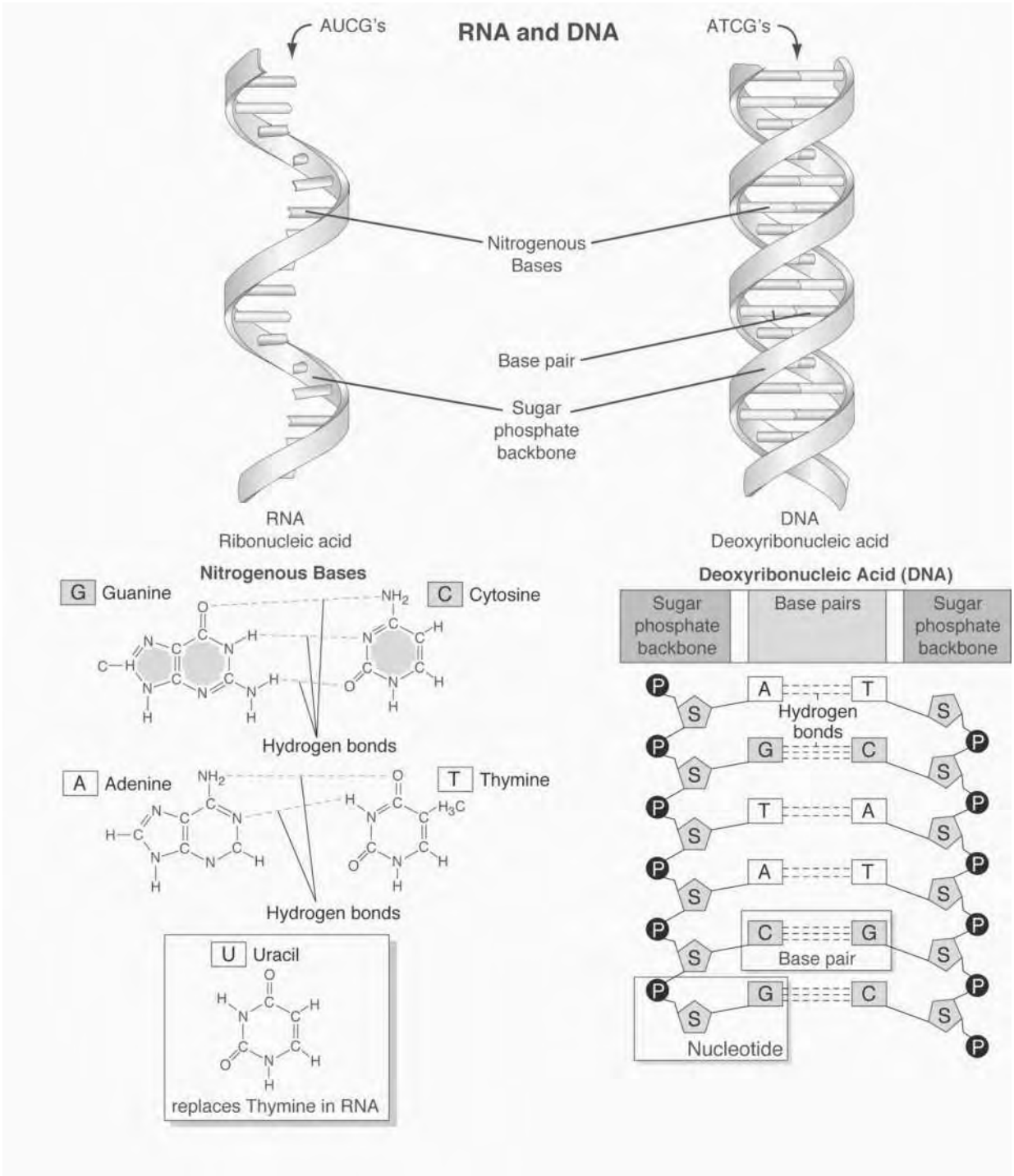


Diagram showing specific base pairing found in DNA and RNA.

them combines with one of the riboses of the backbone to form a nucleoside, such as adenosine, guanosine, cytidine, and uridine. The combination of a ribose, a phosphate, and a given nitrogenous base by its turn results in a nucleotide, such as adenylate, guanylate, cytidylate, uridylate. Each phosphodiester bridge links the 3' carbon at the ribose of one nucleotide to the 5' carbon at the ribose of the subsequent nucleotide, and so on. RNA molecules fold on themselves and form structures termed hairpin loops, because they have extensive regions of complementary guanine-cytosine (G-C) or adenine-uracil (A-U) pairs. Nevertheless, they are single polynucleotide chains.

The mRNA molecules contain at the 5' end a leader sequence that is not translated, known as UTR (untranslated region) and an initiation codon (AUG), that precedes the coding region formed by the spliced exons, which are termed codons in the mature mRNA. At the end of the coding region, three termination codons (UAG, UAA, UGA) are present, being followed by a trailer sequence that constitutes another UTR, which is by its turn followed by the poly-A tail. The stability of the mRNA molecule is crucial to the proper translation of the transcript into protein. The poly-A tail is responsible by such stability because it prevents the precocious degradation of mRNA by a 3' to 5' exonuclease (a cytoplasmatic enzyme that digests mRNA starting from the extremity 3' when the molecule leaves the cell nucleus). The mRNA of histones, the nuclear proteins that form the nucleosomes, do not have poly-A tails, thus constituting an exception to this rule. The poly-A tail also protects the other extremity of the mRNA molecule by looping around and touching the 7-methylguanosine cap attached to the 5' extremity. This prevents the decapping of the mRNA molecule by another exonuclease. The removal of the 7-methylguanosine exposes the 5' end of the mRNA to digestion by the 5' to 3' exonuclease (a cytoplasmatic enzyme that digests mRNA starting from the 5' end). When the translation of the protein is completed, the enzymatic process of deadenylation (i.e., enzymatic digestion of the poly-A tail) is activated, thus allowing the subsequent mRNA degradation by the two above mentioned exonucleases, each working at one of the ends of the molecule.

Transfer RNA (tRNA) is often referred to as the "Rosetta Stone" of genetics, as it translates the instructions encoded by DNA, by way of messenger RNA (mRNA), into specific sequences of amino acids that form proteins and polypeptides. This class of small globular RNA is only 75 to 90 nucleotides long, and there is at least one tRNA for every amino acid. The job of tRNA is to transport free amino acids within the cell and attach them to the growing polypeptide chain. First, an amino acid molecule is attached to its particular tRNA. This process is catalyzed by an enzyme called aminoacyl—tRNA synthetase that binds to the inside of the tRNA molecule. The molecule is now charged. The next step, joining the amino acid to the polypeptide chain, is carried out inside the ribosome. Each amino acid is specified by a particular sequence of three nucleotide bases called codons. There are four different kinds of nucleotides in mRNA. This makes possible 64 different codons (4^3). Two of these codons are called STOP codons; one of these is the START codon (AUG). With only 20 different amino acids, it is clear that some amino acids have more then one codon. This is referred to as the degeneracy of the genetic code. On the other end of the tRNA molecule are three special nucleotide bases called the anticodon. These interact with three complimentary codon bases in the mRNA by way of hydrogen bonds. These weak directional bonds are also the force that holds together the double strands of DNA.

In order to understand how this happens, it was necessary to first understand the three dimensional structure (conformation) of the tRNA molecule. This was first attempted in 1965, where the two-dimensional folding pattern was deduced from the sequence of nucleotides found in **yeast** alanine tRNA. Later work (1974), using x-ray diffraction analysis, was able to reveal the conformation of yeast phenylalanine tRNA. The molecule is shaped like an upside-down L. The vertical portion is made up of the D stem and the anti-codon stem, and the horizontal arm of the L is made up of the acceptor stem and the T stem. Thus, the translation depends entirely upon the physical structure. At one end of each tRNA is a structure that recognizes the genetic code, and at the other end is the particular amino acid for that code. Amazingly, this unusual shape is conserved between **bacteria**, plants, and animals.

Another unusual thing about tRNA is that it contains some unusual bases. The other classes of nucleic acids can undergo the simple modification of adding a methyl (CH_3^-) group. However, tRNA is unique in that it undergoes a range of modifications from methylation to total restructuring of the purine ring. These modifications occur in all parts of the tRNA molecule, and increase its structural integrity and versatility.

Ribosomes are composed of ribosomal RNA (as much as 50%) and special proteins called ribonucleoproteins. In **eukaryotes** (an organism whose cells have chromosomes with nucleosomal structure and are separated from the cytoplasm by a two membrane nuclear envelope and whose functions are compartmentalized into distinct cytoplasmic organelles), there are actually four different types of rRNA. One of these molecules is called 18SrRNA; along with some 30–plus different proteins, it makes up the small subunit of the ribosome. The other three types of rRNA are called 28S, 5.8S, and 5S rRNA. One of each of these molecules, along with some 45 different proteins, is used to make the large subunit of the ribosome. There are also two rRNAs exclusive to the mitochondrial (a circular molecule of some 16,569 base pairs in the human) genome. These are called 12S and 16S. A mutation in the 12SrRNA has been implicated in non-syndromic hearing loss. Ribosomal RNA's have these names because of their molecular weight. When rRNA is spun down by ultracentrifuge, these molecules sediment out at different rates because they have different weights. The larger the number, the larger the molecule.

The larger subunit appears to be mainly involved in such biochemical processes as catalyzing the reactions of polypeptide chain elongation and has two major binding sites. Binding sites are those parts of large molecule that actively participate in its specific combination with another molecule. One is called the aminoacyl site and the other is called the peptidyl site. Ribosomes attach their peptidyl sites to the membrane surface of the rER. The aminoacyl site has been associated with binding transfer RNA. The smaller subunit appears to be concerned with ribosomal recognition processes such as mRNA. It is involved with the binding of tRNA also. The smaller subunit combines with mRNA and the first "charged " tRNA to form the initiation complex for translation of the RNA sequence into the final polypeptide.

The precursor of the 28S, 18S and the 5.83S molecules are transcribed by RNA polymerase I (Pol I) and the 5S rRNA is transcribed by RNA polymerase III (PoIII). Pol I is the most active of all the RNA polynmerases, and is one indication of how important these structures are to cellular function.

Ribosomal RNAs fold in very complex ways. Their structure is an important clue to the evolutionary relationships found between different kinds of organisms. Sequence comparisons of the various rRNAs across various species show that even though their base sequences vary widely, **evolution** has conserved their secondary structures, therefore, organization must be important for their function.

Since the 1970s, nucleic acids' cellular processes have become the basis for genetic engineering, in which scientists add or remove genes in order to alter the characteristics or behavior of cells. Such techniques are used in agriculture, pharmaceutical and other chemical manufacturing, and medical treatments for cancer and other diseases.

See also Biochemistry; Genetic regulation of eukaryotic cells

RIBOSOMES

Ribosomes are organelles that play a key role in the manufacture of proteins. Found throughout the cell, ribosomes are composed of ribosomal **ribonucleic acid** (rRNA) and proteins. They are the sites of **protein synthesis**.

Although **Robert Hooke** first used a light **microscope** to look at cells in 1665, it was only during the last few decades that the cell's organelles were discovered. This is primarily because light microscopes do not have the magnifying power required to see these tiny structures. Using an **electron microscope**, scientists have been able to see most of the cells substructures, including the ribosomes.

Ribosomes are composed of a variety of proteins and rRNA. They are organized in two functional subunits that are constructed in the cell's nucleolus. One is a small subunit that has a squashed shape, while another is a large subunit that is spherical in shape. The large subunit is about twice as big as the small unit. The subunits usually exist separately, but join when they are attached to a messenger **RNA** (mRNA). This initiates protein synthesis.

Production of a protein begins with initiation. In this step, the ribosomal small subunit binds to the mRNA along with the first transfer RNA (tRNA). The next step is elongation, where the ribosome moves along the mRNA and strings together the amino acids one by one. Finally, the ribosome encounters a stop sequence and the two subunits release the mRNA, the polypeptide chain, and the tRNA.

Protein synthesis occurs at specific sites within the ribosome. The P site of a ribosome contains the growing protein chain. The A site holds the tRNA that has the next amino acid. The two sites are held close together and a chemical reaction occurs. When the stop signal is present on the mRNA, protein synthesis halts. The polypeptide chain is released and the ribosome subunits are returned to the pool of ribosome units in the **cytoplasm**.

Ribosomes are found in two locations in the cell. Free ribosomes are dispersed throughout the cytoplasm. Bound ribosomes are attached to a membranous structure called the endoplasmic reticulum. Most cell proteins are made by the free ribosomes. Bound ribosomes are instrumental in producing proteins that function within or across the cell membrane. Depending on the cell type, there can be as many as a few million ribosomes in a single cell.

Because most cells contain a large number of ribosomes, rRNA is the most abundant type of RNA. rRNA plays an active role in ribosome function. It interacts with both the mRNA and tRNA and helps maintain the necessary structure. Transfer RNA is the molecule that interacts with the mRNA during protein synthesis and is able to read a three amino acid sequence. On the opposite end of the tRNAs, amino acids are bonded on a growing polypeptide chain. Generally, it takes about a minute for a single ribosome to make an average sized protein. However, several ribosomes can work on a single mRNA at the same time. This allows the cell to make many copies of a single protein rapidly. Sometimes these multiple ribosomes, or polysomes, can become so large that they can be seen with a light microscope.

The ribosomes in **eukaryotes** and prokaryotes are slightly different. Eukaryotic ribosomes are generally larger and are made up of more proteins. Since many diseases are caused by prokaryotes, these slight differences have important medical implications. Drugs have been developed that can inhibit the function of a prokaryotic ribosome, but leave the eukaryotic ribosome unaffected. One example is the antibiotic tetracycline.

See also Protein synthesis

RICKETTSIA AND RICKETTSIAL POX

Rickettsia are a group of **bacteria** that cause a number of serious human diseases, including the spotted fevers and **typhus**. Rod- or sphere-shaped, rickettsia lack both flagella (whip-like organs that allow bacteria to move) and pili (short, flagella-like projections that help bacteria adhere to host cells). Specific species of rickettsia include *Rickettsia rickettsii,* which causes the dangerous Rocky Mountain spotted fever; *R. akari,* which causes the relatively mild rickettsial pox; *R. prowazekii,* which causes the serious disease epidemic typhus; *R. typhi,* the cause of the more benign endemic or rat typhus; and *R. tsutsugamushi,* the cause of scrub typhus.

Rickettsia are transmitted to humans by insects such as ticks, mites, and chiggers. Usually the insect has acquired the bacteria from larger animals which they parasitize, such as rats, mice, and even humans. When an insect infected with rickettsia bites a human, the bacteria enter the bloodstream. From there, unlike most other bacteria that cause infection by adhering to cells, rickettsia enter specific human cells, where they reproduce. Eventually these host cells lyse (burst open), releasing more rickettsia into the bloodstream. Most rickettsial diseases are characterized by fever and a rash. Although all can be effectively cured with **antibiotics**, some of the rickettsial diseases, such as epidemic typhus and Rocky Mountain spotted fever, can be fatal if not treated promptly.

Rocky Mountain spotted fever is one of the most severe rickettsial diseases. First recognized in the Rocky Mountains, it has since been found to occur throughout the United States.

The **Centers for Disease Control** report about 600–1,000 cases occurring annually, but this number may be underestimated due to underreporting. *Rickettsia rickettsii* are carried and transmitted by four species of the hard-shelled tick, all of which feed on humans, wild and domestic animals, and small rodents. When a tick feeds on an infected animal, the bacteria are transmitted to the tick, which can in turn infect other animals with its bite. Human-to-human transmission of *R. rickettsii* does not occur. Once inside the human bloodstream, the bacteria invade cells that line the small blood vessels.

The symptoms of Rocky Mountain spotted fever reflect the presence of bacteria inside blood vessel cells. Within two to 12 days of being bitten by an infected tick, the infected person experiences a severe headache, fever, and malaise. After about two to four days, a rash develops, first on the extremities, then the trunk. A characteristic sign of this disease is that the rash involves the soles of the feet and palms of the hands. If the disease is not treated with antibiotics, the infected blood vessel cells lyse, causing internal hemorrhage, blockage of the blood vessels, and eventual death of the cells. Shock, kidney failure, heart failure, and stroke may then occur. Rocky Mountain spotted fever is often fatal if not treated.

A similar but milder disease is rickettsial pox, caused by *R. akari*. These bacteria are transmitted by mites which live preferentially on the common house mouse, only occasionally biting humans. Rickettsial pox is characterized by a rash that does not affect the palms or soles of the feet. The rash includes a lesion called an eschar-a sore that marks the spot of the infected mite bite. The mild course of this disease and the presence of the rash sometimes leads to its misdiagnosis as chicken pox, but the eschar clearly distinguishes rickettsial pox from chicken pox.

Outside of the United States, spotted fevers such as North Asian tick typhus, Queensland tick typhus, and boutonneuse fever are caused by other rickettsia species. As their names suggest, these diseases are found in Asia, Mongolia, and the Siberian region of Russia; in Australia; and in the Mediterranean region, Africa, and India, respectively. Symptoms of these spotted fevers resemble those of rickettsial pox. Although these spotted fevers share some of the symptoms of Rocky Mountain spotted fever, they are milder diseases and are usually not fatal.

Three forms of typhus are also caused by rickettsia. Epidemic typhus is caused by *R. prowazekii,* a bacterium that is transmitted by the human body louse. Consequently, episodes of this disease occur when humans are brought into close contact with each other under unsanitary conditions. Endemic typhus and scrub typhus are caused by *R. typhi* and *R. tsutsugamushi,* respectively. Transmitted by rat fleas, endemic typhus is a mild disease of fever, headache, and rash. Scrub typhus, named for its predilection for scrub habitats (although it has since been found to occur in rain forests, savannas, beaches, and deserts as well) is transmitted by chiggers. Unlike endemic typhus, scrub typhus is a serious disease that is fatal if not treated.

Not all rickettsia cause disease. Some species, such as *R. parkeri* and *R. montana,* normally live inside certain species of ticks and are harmless to the insect. These rickettsia are nonpathogenic (they do not cause disease) to humans as well.

With the exception of epidemic typhus, no **vaccine** exists to prevent rickettsial infection. Prevention of these diseases should focus on the elimination of insect carriers with insecticides and wearing heavy clothing when going into areas in which rickettsial carriers dwell. For instance, appropriate clothing for a forest expedition should include boots, long-sleeved shirts, and long pants. Treating the skin with insect **repellents** is also recommended to prevent insect bites.

RNA TUMOR VIRUSES

RNA tumor viruses contain **ribonucleic acid** as their genetic material. The **viruses** derive their designation from their association with tumors.

RNA tumor viruses are **retroviruses** that possess the reverse transcriptase enzyme that manufactures **deoxyribonucleic acid** (**DNA**) from the RNA template. Indeed, *retro* is the Latin word for backwards.

The history of RNA tumor viruses extends back to the first decade of the twentieth century. In 1908, it was demonstrated that fluid from a chicken that has leukemia could cause cells to be cancerous, even after the fluid had been filtered to remove all bacteria-sized organisms. Three years later, **Peyton Rous** identified one such factor, now named the Rous Sarcoma Virus. By the 1950s, many oncogenic viruses had been discovered and the RNA nature of these viruses had been established. In the 1960s, the reverse transcriptase enzyme was discovered. Finally, in 1981, the first human retrovirus was discovered, this being the **Human T-cell Leukemia Virus** (HTLV-1). The latter virus is a well-known tumor viruses.

There are two groups of RNA tumor viruses, the Oncovirinae and the Lentivirinae. Examples of the first group include the Rous Sarcoma Virus, HTLV-1, and HTLV-2 (which is also known as hairy cell leukemia virus). A prominent example of the second group is the **Human Immunodeficiency Virus** (**HIV**). A characteristic of HIV and other members of the second group is the long period of latency before symptoms of infection appear.

As for many viruses, the investigation of RNA tumor viruses involves growing the virus in a **culture** of whatever eukaryotic cell the virus is able to replicate inside. Then, the virus is purified. Subsequently, the virus can be studied using a variety of molecular and genetic techniques, and the **electron microscope** to assess the shape of the virus particles.

Some RNA tumor viruses never exist outside of the host cell, and lack an envelope around their genetic material. Viruses such as the mouse mammary tumor virus have an envelope that has spikes protruding from the surface. Other RNA tumor viruses contain spikes that are less prominent. Lentiviruses are an example of the latter shape.

The envelope of RNA tumor viruses comes from the membrane surrounding the host cell. The virus acquires this envelope as it emerges from the host cell. Within this envelope are distinctive proteins, which are coded for by the envelope, or env, **gene**. Another characteristic component of RNA

tumor viruses is the presence of a protein that coats the viral RNA. The gag gene codes for this latter protein. The protein encoded by the gag gene is also found in the envelope. The presence of these two protein species in RNA tumor viruses is being explored as a target for therapy to prevent RNA virus-induced cancer.

Another hallmark of RNA tumor viruses is the presence of a gene that is designated pol. The products of the pol gene include reverse transcriptase, another enzyme that helps integrate the viral genetic material into the host genome, and other **enzymes** that help process the genetic material and viral proteins so as to permit assembly of new virus. These essential functions have made the pol gene the target of antiviral strategies.

The infection process begins with the binding of the virus particles to a specific molecule on the surface of the host cell. Generically, such molecules are termed receptors. Once the virus is bound, it can be taken into the host by the process of endocytosis. Blocking the viral recognition of the host receptor and binding of the virus is yet another strategy to prevent tumor development.

The molecular basis for the **transformation** of cells by RNA tumor viruses was revealed by a number of scientists, including the Nobel laureate Harold Varmus. He and the others demonstrated that the cancer genes (oncogenes) of the viruses were similar or the same as certain genes with the nucleic acid of the host cell. When a virus infects the host, the host gene may become part of a new virus particle following viral replication. Over time, the host gene may become altered in subsequent rounds of viral replication. Eventually, this altered host gene may end up replacing a normal gene in a new host cell. The altered gene produces a protein that is involved in over-riding the controls on the division process of the host cell. The result is the uncontrolled cell division that is the hallmark of a cancer cell.

See also AIDS, recent advances in research and treatment; Immunodeficiency; Viral genetics

ROUS, PEYTON (1879-1970)

American physician

Francis Peyton Rous was a physician-scientist at the Rockefeller Institute for Medical Research (later the Rockefeller University) for over sixty years. In 1966, Rous won the Nobel Prize for his 1910 discovery that a virus can cause cancer tumors. His other contributions to scientific medicine include creating the first blood bank, determining major functions of the liver and gall bladder, and identifying factors that initiate and promote malignancy in normal cells.

Rous was born in Baltimore, Maryland, to Charles Rous, a grain exporter, and Frances Wood, the daughter of a Texas judge. His father died when Rous was eleven, and his mother chose to stay in Baltimore. His sisters were professionally successful, one a musician, the other a painter.

Rous, whose interest in natural science was apparent at an early age, wrote a "flower of the month" column for the *Baltimore Sun*. He pursued his biological interests at Johns Hopkins University, receiving a B.A. in 1900 and an M.D. in 1905. After a medical internship at Johns Hopkins, however, he decided (as recorded in *Les Prix Nobel en 1966*) that he was "unfit to be a real doctor" and chose instead to concentrate on research and the natural history of disease. This led to a full year of studying lymphocytes with Aldred Warthin at the University of Michigan and a summer in Germany learning morbid anatomy (pathology) at a Dresden hospital.

After Rous returned to the United States, he developed pulmonary **tuberculosis** and spent a year recovering in an Adirondacks sanatorium. In 1909, Simon Flexner, director of the newly founded Rockefeller Institute in New York City, asked Rous to take over cancer research in his laboratory. A few months later, a poultry breeder brought a Plymouth Rock chicken with a large breast tumor to the Institute and Rous, after conducting numerous experiments, determined that the tumor was a spindle-cell sarcoma. When Rous transferred a cell-free filtrate from the tumor into healthy chickens of the same flock, they developed identical tumors. Moreover, after injecting a filtrate from the new tumors into other chickens, a malignancy exactly like the original formed. Further studies revealed that this filterable agent was a virus, although Rous carefully avoided this word. Now called the Rous sarcoma virus RSV) and classed as an **RNA** retrovirus, it remains a prototype of animal **tumor viruses** and a favorite laboratory model for studying the role of genes in cancer.

Rous's discovery was received with considerable disbelief, both in the United States and in the rest of the world. His viral theory of cancer challenged all assumptions, going back to Hippocrates, that cancer was not infectious but rather a spontaneous, uncontrolled growth of cells and many scientists dismissed his finding as a disease peculiar to chickens. Discouraged by his failed attempts to cultivate **viruses** from mammal cancers, Rous abandoned work on the sarcoma in 1915. Nearly two decades passed before he returned to cancer research.

After the onset of World War I, Rous, J. R. Turner, and O. H. Robertson began a search for emergency blood transfusion fluids. Nothing could be found that worked without red blood corpuscles so they developed a citrate-sugar solution that preserved blood for weeks as well as a method to transfuse the suspended cells. Later, behind the front lines in Belgium and France, they created the world's first blood bank from donations by army personnel. This solution was used again in World War II, when half a million Rous-Turner blood units were shipped by air to London during the Blitz.

During the 1920s, Rous made several contributions to physiology. With P. D. McMaster, Rous demonstrated the concentrating activity of bile in the gall bladder, the acid-alkaline balance in living tissues, the increasing permeability along capillaries in muscle and skin, and the nature of gallstone formation. In conducting these studies, Rous devised **culture** techniques that have become standard for studying living tissues in the laboratory. He originated the method for growing viruses on chicken embryos, now used on a mass scale for producing viral vaccines, and found a way to isolate single cells from solid tissues by using the enzyme trypsin.

Moreover, Rous developed an ingenious method for obtaining pure cultures of Kupffer cells by taking advantage of their phagocytic ability; he injected iron particles in animals and then used a magnet to separate these iron-laden liver cells from suspensions.

In 1933, a Rockefeller colleague's report stimulated Rous to renew his work on cancer. Richard Shope discovered a virus that caused warts on the skin of wild rabbits. Within a year, Rous established that this papilloma had characteristics of a true tumor. His work on mammalian cancer kept his viral theory of cancer alive. However, another twenty years passed before scientists identified viruses that cause human cancers and learned that viruses act by invading genes of normal cells. These findings finally advanced Rous's 1910 discovery to a dominant place in cancer research.

Meanwhile, Rous and his colleagues spent three decades studying the Shope papilloma in an effort to understand the role of viruses in causing cancer in mammals. Careful observations, over long periods of time, of the changing shapes, colors, and sizes of cells revealed that normal cells become malignant in progressive steps. Cell changes in tumors were observed as always evolving in a single direction toward malignancy.

The researchers demonstrated how viruses collaborate with carcinogens such as tar, radiation, or chemicals to elicit and enhance tumors. In a report co-authored by W. F. Friedewald, Rous proposed a two-stage mechanism of carcinogenesis. He further explained that a virus can be induced by carcinogens or it can hasten the growth and transform benign tumors into cancerous ones. For tumors having no apparent trace of virus, Rous cautiously postulated that these spontaneous growths might contain a virus that persists in a masked or latent state, causing no harm until its cellular environment is disturbed.

Rous eventually ceased his research on this project due to the technical complexities involved with pursuing the interaction of viral and environmental factors. He then analyzed different types of cells and their nature in an attempt to understand why tumors go from bad to worse.

Rous maintained a rigorous workday schedule at Rockefeller. His meticulous editing and writing, both scientific and literary, took place during several hours of solitude at the beginning and end of each day. At midday, he spent two intense hours discussing science with colleagues in the Institute's dining room. Rous then returned to work in his laboratory on experiments that often lasted into the early evening.

Rous was appointed a full member of the Rockefeller Institute in 1920 and member emeritus in 1945. Though officially retired, he remained active at his lab bench until the age of ninety, adding sixty papers to the nearly three hundred he published. He was elected to the National Academy of Sciences in 1927, the American Philosophical Society in 1939, and the Royal Society in 1940. In addition to the 1966 Nobel Prize for Medicine, Rous received many honorary degrees and awards for his work in viral oncology, including the 1956 Kovalenko Medal of the National Academy of Sciences, the 1958 Lasker Award of the American Public Health Association, and the 1966 National Medal of Science.

As editor of the *Journal of Experimental Medicine,* a periodical renowned for its precise language and scientific excellence, Rous dominated the recording of forty-eight years of American medical research. He died of abdominal cancer in New York City, just six weeks after he retired as editor.

See also Viral genetics; Viral vectors in gene therapy; Virology; Virus replication; Viruses and responses to viral infection

ROUX, PIERRE-PAUL-ÉMILE (1853-1933)

French physician and bacteriologist

Soon after becoming a doctor, Émile Roux began doing research on bacterial diseases for **Louis Pasteur**. It has taken a century, however, for Roux's contribution to Pasteur's work—specifically his experiments utilizing dead **bacteria** to vaccinate against rabies—to be acknowledged. Roux is also credited, along with Alexandre Yersin, with the discovery of the **diphtheria** toxin secreted by *Corynebacterium diphtheriae* and **immunization** against the disease in humans. Both colleague and close friend to Pasteur, Roux eventually became the director of the Pasteur Institute in Paris.

Roux began his study of medicine at the Clermont-Ferrand Medical School in 1872. In 1874 Roux moved to Paris where he continued his studies at a private clinic. In 1878 he helped create lectures on **fermentation** for Emile Duclaux at the Sorbonne, Paris. Duclaux introduced Roux to Louis Pasteur, who was then in need of a doctor to assist with his research on bacterial diseases.

In 1879 Roux first began assisting Pasteur on his experiments with chicken cholera. The cholera bacillus was grown in pure **culture** and then injected into chickens, which would invariably die within 48 hours. However, one batch of culture was left on the shelf too long and when injected into chickens, failed to kill them. Later, these same chickens—in addition to a new group of chickens—were injected with new cultures of the cholera bacillus. The new group of chickens died while the first group of chickens remained healthy. Thus began the studies of the attenuation of chicken cholera.

In the 1880's Pasteur and Roux began research on rabid animals in hopes of finding a **vaccine** for **rabies**. Pasteur proceeded by inoculating dogs with an attenuated (weakened) strain of the bacteria from the brain tissue of rabid animals. Roux worked on a similar experiment utilizing dead rather than weakened bacteria from the dried spinal cords of infected rabbits.

On July 4, 1885, a 9-year-old boy named Joseph Meister was attacked on his way to school and repeatedly bitten by a rabid dog. A witness to the incident rescued Meister by beating the dog away with an iron bar; the dog's owner, Theodore Vone, then shot the animal. Meister's wounds were cauterized with carbolic acid and he was taken to a local doctor. This physician realized that Meister's chance of survival was minimal and suggested to Meister's mother that she take her son to Paris to see Louis Pasteur, who had successfully vaccinated dogs against rabies. The vaccine had never been

tried on humans, and Pasteur was reluctant to give it to the boy; but when two physicians stated that Meister would die without it, Pasteur relented and administered the vaccine.

Pasteur stated that he utilized the attenuated strain of the vaccine; his lab notes, however, confirm that he treated Meister with the dead strain that Roux had been working on. (Why Pasteur maintained that he used his attenuated strain is not clear.) In any case, Meister received 13 shots of the rabies vaccine in the stomach in 10 days and was kept under close observation for an additional 10 days. The boy survived and became the first person to be immunized against rabies.

In 1883 Roux became the assistant director of Pasteur's laboratory. He undertook administrative responsibilities to help establish the Pasteur Institute, which opened in 1888 with Roux serving as director (from 1904) and teaching a class in microbiology.

Also in 1883 Roux and Yersin discovered the diphtheria toxin secreted by *Corynebacterium diphtheriae*. The two scientists filtered the toxin from cultures of the diphtheria bacterium and injected it into healthy laboratory animals. The animals exhibited the same symptoms (and eventual death) as those infected with the bacterium. Other data to support their discovery of the diphtheria toxin included urine obtained from children infected with the microorganism. Toxin excreted in the urine was sufficient to produce the same symptoms of the disease in laboratory animals. In 1894 Roux and Louis Martin began to study the immunization of horses against diphtheria in order to create a serum to be used in humans. The outcome of their research led them to successfully treat 300 children with the serum.

Beginning in 1896 Roux researched different aspects of diseases such as **tetanus**, **tuberculosis**, bovine **pneumonia**, and **syphilis** until he became the director of the Pasteur Institute in 1904. At that time Roux ceased all personal research and focused solely on running the Pasteur Institute until his death from tuberculosis in 1933.

See also Bacteria and bacterial infection; History of microbiology; History of public health

RUSKA, ERNST (1906-1988)

German physicist

The inventor of the **electron microscope**, Ernst Ruska, combined an academic career in physics and electrical engineering with work in private industry at several of Germany's top electrical corporations. He was associated with the Siemens Company from 1937 to 1955, where he helped mass produce the electron **microscope**, the invention for which he was awarded the 1986 Nobel Prize in physics. The Nobel Prize Committee called Ruska's electron microscope one of the most important inventions of the twentieth century. The benefits of electron microscopy to the field of microbiology and medicine allow scientists to study such structures as **viruses** and protein molecules. Technical fields such as electronics have also found new uses for Ruska's invention: improved versions of the electron microscope became instrumental in the fabrication of computer chips.

Ruska was born in Heidelberg, Germany, on December 25, 1906. He was the fifth child of Julius Ferdinand Ruska, an Asian studies professor, and Elisabeth (Merx) Ruska. After receiving his undergraduate education in the physical sciences from the Technical University of Munich and the Technical University of Berlin, he was certified as an electrical engineer in 1931. He then went on to study under Max Knoll at Berlin, and received his doctorate in electrical engineering in 1933. During this period, Ruska and Knoll created an early version of the electron microscope, and Ruska concurrently was employed by the Fernseh Corporation in Berlin, where he worked to develop television tube technology. He left Fernseh to join Siemens as an electrical engineer, and at the same time accepted a position as a lecturer at the Technical University of Berlin. His ability to work in both academic and corporate milieus continued through his time at Siemens, and expanded when in 1954, he became a member of the Max Planck Society. In 1957, he was appointed director of the Society's Institute of Electron Microscopy, and in 1959, he accepted the Technical University of Berlin's invitation to become professor of electron optics and electron microscopy. Ruska remained an active contributor to his field until his retirement in 1972.

Prior to Ruska's invention of the electron microscope in 1931, the field of microscopy was limited by the inability of existing microscopes to see features smaller than the wavelength of visible light. Because the wavelength of light is about two thousand times larger than an atom, the mysteries of the atomic world were virtually closed to scientists until Ruska's breakthrough using electron wavelengths as the resolution medium. When the electron microscope was perfected, microscope magnification increased from approximately two thousand to one million times.

The French physicist, **Louis Victor de Broglie**, was the first to propose that subatomic particles, such as electrons, had wavelike characteristics, and that the greater the energy exhibited by the particle, the shorter its wavelength would be. De Broglie's theory was confirmed in 1927 by Bell Laboratory researchers. The conception that it was possible to construct a microscope that used electrons instead of light was realized in the late 1920s when Ruska was able to build a short-focus magnetic lens using a magnetic coil. A prototype of the electron microscope was then developed in 1931 by Ruska and Max Knoll at the Technical University in Berlin. Although it was less powerful than contemporary optical microscopes, the prototype laid the groundwork for a more powerful version, which Ruska developed in 1933. That version was ten times stronger than existing light microscopes. Ruska subsequently worked with the Siemens Company to produce for the commercial market an electron microscope with a resolution to one hundred angstroms (by contrast, modern electron microscopes have a resolution to one angstrom, or one ten-billionth of a meter).

Ruska's microscope—called a transmission microscope—captures on a fluorescent screen an image made by a focused beam of electrons passing through a thin slice of metalized material. The image can be photographed. In 1981,

Gerd Binnig and Heinrich Rohrer took Ruska's concept further by using a beam of electrons to scan the surface of a specimen (rather than to penetrate it). A recording of the current generated by the intermingling of electrons emitted from both the beam and specimen is used to build a contour map of the surface. The function of this scanning electron microscope complements, rather than competes against, the transmission microscope, and its inventors shared the 1986 Nobel Prize in physics with Ruska.

In 1937, Ruska married Irmela Ruth Geigis, and the couple had two sons and a daughter. In addition to the Nobel Prize, Ruska's work was honored with the Senckenberg Prize of the University of Frankfurt am Main in 1939, the Lasker Award in 1960, and the Duddell Medal and Prize of the Institute of Physics in London in 1975, among other awards. He also held honorary doctorates from the University of Kiev, the University of Modena, the Free University of Berlin, and the University of Toronto. Ruska died in West Berlin on May 30, 1988.

See also Microscope and microscopy

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S LAYER • *see* SHEATHED BACTERIA

SABIN, ALBERT (1906-1993)

Russian American virologist

Albert Sabin developed an oral **vaccine** for polio that led to the once-dreaded disease's virtual extinction in the Western Hemisphere. Sabin's long and distinguished research career included many major contributions to **virology**, including work that led to the development of attenuated live-virus vaccines. During World War II, he developed effective vaccines against **dengue fever** and Japanese B encephalitis. The development of a live polio vaccine, however, was Sabin's crowning achievement.

Although Sabin's polio vaccine was not the first, it eventually proved to be the most effective and became the predominant mode of protection against polio throughout the Western world. In South America, "Sabin Sundays" were held twice a year to eradicate the disease. The race to produce the first effective vaccine against polio was marked by intense and often acrimonious competition between scientists and their supporters; in addition to the primary goal of saving children, fame and fortune were at stake. Sabin, however, allowed his vaccine to be used free of charge by any reputable organizations as long as they met his strict standards in developing the appropriate strains.

Albert Bruce Sabin was born in Bialystok, Russia (now Poland), on August 26, 1906. His parents, Jacob and Tillie Sabin, immigrated to the United States in 1921 to escape the extreme poverty suffered under the czarist regime. They settled in Paterson, New Jersey, and Sabin's father became involved in the silk and textile business. After Albert Sabin graduated from Paterson High School in 1923, one of his uncles offered to finance his college education if Sabin would agree to study dentistry. Later, during his dental education, Sabin read the *Microbe Hunters* by Paul deKruif and was drawn to the science of virology, as well as to the romantic and heroic vision of conquering epidemic diseases.

After two years in the New York University (NYU) dental school, Sabin switched to medicine and promptly lost his uncle's financial support. He paid for school by working at odd jobs, primarily as a lab technician and through scholarships. He received his B.S. degree in 1928 and enrolled in NYU's College of Medicine. In medical school, Sabin showed early promise as a researcher by developing a rapid and accurate system for typing (identifying) *Pneumococci,* or the **pneumonia viruses**. After receiving his M.D. degree in 1931, he went on to complete his residency at Bellevue Hospital in New York City, where he gained training in pathology, surgery, and internal medicine. In 1932, during his internship, Sabin isolated the B virus from a colleague who had died after being bitten by a monkey. Within two years, Sabin showed that the B virus's natural habitat is the monkey and that it is related to the human **Herpes** Simplex virus. In 1934, Sabin completed his internship and then conducted research at the Lister Institute of Preventive Medicine in London.

In 1935, Sabin returned to the United States and accepted a fellowship at the Rockefeller Institute for Medical Research. There, he resumed in earnest his research of **poliomyelitis** (or polio), a paralytic disease that had reached epidemic proportions in the United States at the time of Sabin's graduation from medical school. By the early 1950s, polio afflicted 13,500 out of every 100 million Americans. In 1950 alone, more than 33,000 people contracted polio. The majority of them were children.

Ironically, polio was once an endemic disease (or one usually confined to a community, group, or region) propagated by poor sanitation. As a result, most children who lived in households without indoor plumbing were exposed early to the virus; the vast majority of them did not develop symptoms and eventually became immune to later exposures. After the **public health** movement at the turn of the century began to improve sanitation and more and more families had indoor toilets, children were not exposed at an early age to

the virus and thus did not develop a natural **immunity**. As a result, polio became an epidemic disease and spread quickly through communities to other children without immunity, regardless of race, creed, or social status. Often victims of polio would lose complete control of their muscles and had to be kept on a respirator, or in a low-pressure iron lung, to help them breathe.

In 1936, Sabin and Peter K. Olitsky used a test tube to grow some poliovirus in the central nervous tissue of human embryos. Not a practical approach for developing the huge amounts of virus needed to produce a vaccine, this research nonetheless opened new avenues of investigation for other scientists. However, their discovery did reinforce the mistaken assumption that polio only affected nerve cells.

Although primarily interested in polio, Sabin was "never able to be a one-virus virologist," as he told Donald Robinson in an interview for Robinson's book *The Miracle Finders*. Sabin also studied how the **immune system** battled viruses and conducted basic research on how viruses affect the central nervous system. Other interests included investigations of **toxoplasmosis**, a usually benign viral disease that sometimes caused death or severe brain and eye damage in prenatal infections. These studies resulted in the development of rapid and sensitive serologic diagnostic tests for the virus.

During World War II, Sabin served in the United States Army Medical Corps. He was stationed in the Pacific theater where he began his investigations into insect-borne encephalitis, sandfly fever, and dengue. He successfully developed a vaccine for dengue fever and conducted an intensive **vaccination** program on Okinawa using a vaccine he had developed at Children's Hospital of Cincinnati that protected more than 65,000 military personnel against Japanese encephalitis. Sabin eventually identified a number of antigenic (or immune response-promoting) types of sandfly fever and dengue viruses that led to the development of several attenuated (avirulent) live-virus vaccines.

After the war, Sabin returned to the University of Cincinnati College of Medicine, where he had previously accepted an appointment in 1937. With his new appointments as professor of research pediatrics and fellow of the Children's Hospital Research Foundation, Sabin plunged back into polio research. Sabin and his colleagues began performing autopsies on everyone who had died from polio within a four-hundred-mile radius of Cincinnati, Ohio. At the same time, Sabin performed autopsies on monkeys. From these observations, he found that the poliovirus was present in humans in both the intestinal tract and the central nervous system. Sabin disproved the widely held assumption that polio entered humans through the nose to the respiratory tract, showing that it first invaded the digestive tract before attacking nerve tissue. Sabin was also among the investigators who identified the three different strains of polio.

Sabin's discovery of polio in the digestive tract indicated that perhaps the polio virus could be grown in a test tube in tissue other than nerve tissue, as opposed to costly and difficult-to-work-with nerve tissue. In 1949, John Franklin Enders, Frederick Chapman Robbins, and Thomas Huckle Sweller grew the first polio virus in human and monkey nonnervous tissue cultures, a feat that would earn them a Nobel Prize. With the newfound ability to produce enough virus to conduct large-scale research efforts, the race to develop an effective vaccine accelerated.

At the same time that Sabin began his work to develop a polio vaccine, a young scientist at the University of Pittsburgh, **Jonas Salk**, entered the race. Both men were enormously ambitious and committed to their own theory about which type of vaccine would work best against polio. While Salk committed his efforts to a killed polio virus, Sabin openly expressed his doubts about the safety of such a vaccine as well as its effectiveness in providing lasting protection. Sabin was convinced that an attenuated live-virus vaccine would provide the safe, long-term protection needed. Such a vaccine is made of living virus that is diluted, or weakened, so that it spurs the immune system to fight off the disease without actually causing the disease itself.

In 1953, Salk seemed to have won the battle when he announced the development of a dead virus vaccine made from cultured polio virus inactivated, or killed, with formaldehyde. While many clamored for immediate mass field trials, Sabin, Enders, and others cautioned against mass inoculation until further efficacy and safety studies were conducted. Salk, however, had won the entire moral and financial support of the National Foundation for Infantile Paralysis, and in 1954, a massive field trial of the vaccine was held. In 1955, to worldwide fanfare, the vaccine was pronounced effective and safe.

Church and town hall bells rang throughout the country, hailing the new vaccine and Salk. However, on April 26, just fourteen days after the announcement, five children in California contracted polio after taking the Salk vaccine. More cases began to occur, with eleven out of 204 people stricken eventually dying. The United States Public Health Service (PHS) ordered a halt to the vaccinations, and a virulent live virus was found to be in certain batches of the manufactured vaccine. After the installation of better safeguards in manufacturing, the Salk vaccine was again given to the public and greatly reduced the incidence of polio in the United States. But Sabin and Enders had been right about the dangers associated with a dead-virus vaccine; and Sabin continued to work toward a vaccine that he believed would be safe, long lasting, and orally administered without the need for injection like Salk's vaccine.

By orally administering the vaccine, Sabin wanted it to multiply in the intestinal tract. Sabin used Enders's technique to obtain the virus and tested individual virus particles on the central nervous system of monkeys to see whether the virus did any damage. According to various estimates, Sabin's meticulous experiments were performed on anywhere from nine to fifteen thousand monkeys and hundreds of chimpanzees. Eventually, he diluted three mutant strains of polio that seemed to stimulate **antibody** production in chimpanzees. Sabin immediately tested the three strains on himself and his family, as well as research associates and volunteer prisoners from Chillicothe Penitentiary in Ohio. Results of these tests showed that the viruses produced

immunity to polio with no harmful side effects. By this time, however, the public and much of the scientific community were committed to the Salk vaccine. Two scientists working for Lederle Laboratories had also developed a live-virus vaccine. However, the Lederle vaccine was tested in Northern Ireland in 1956 and proved dangerous, as it sometimes reverted to a virulent state.

Although Sabin lacked backing for a large-scale clinical trial in the United States, he remained undaunted. He was able to convince the Health Ministry in the Soviet Union to try his vaccine in massive trials. At the time, the Soviets were mired in a polio epidemic that was claiming eighteen to twenty thousand victims a year. By this time, Sabin was receiving the political backing of the **World Health Organization** in Geneva, Switzerland, which had previously been using Salk's vaccine to control the outbreak of polio around the world; they now believed that Sabin's approach would one day eradicate the disease.

Sabin began giving his vaccine to Russian children in 1957, inoculating millions over the next several years. Not to be outdone by Salk's public relations expertise, Sabin began to travel extensively, promoting his vaccine through newspaper articles, issued statements, and scientific meetings. In 1960, the U.S. Public Health Service, finally convinced of Sabin's approach, approved his vaccine for manufacture in the United States. Still, the PHS would not order its use and the Salk vaccine remained the vaccine of choice until a pediatrician in Phoenix, Arizona, Richard Johns, organized a Sabin vaccine drive. The vaccine was supplied free of charge, and many physicians provided their services without a fee on a chosen Sunday. The success of this effort spread, and Sabin's vaccine soon became "the vaccine" to ward off polio.

The battle between Sabin and Salk persisted well into the 1970s, with Salk writing an op-ed piece for the New York Times in 1973 denouncing Sabin's vaccine as unsafe and urging people to use his vaccine once more. For the most part, Salk was ignored, and by 1993, health organizations began to report that polio was close to extinction in the Western Hemisphere.

Sabin continued to work vigorously and tirelessly into his seventies, traveling to Brazil in 1980 to help with a new outbreak of polio. He antagonized Brazilian officials, however, by accusing the government bureaucracy of falsifying data concerning the serious threat that polio still presented in that country. He officially retired from the National Institute of Health in 1986. Despite his retirement, Sabin continued to be outspoken, saying in 1992 that he doubted whether a vaccine against the **human immunodeficiency virus**, or **HIV**, was feasible. Sabin died from congestive heart failure at the Georgetown University Medical Center on March 3, 1993. In an obituary in the *Lancet,* Sabin was noted as the "architect" behind the eradication of polio from North and South America. Salk issued a statement praising Sabin's work to vanquish polio.

See also Antibody and antigen; Antibody formation and kinetics; History of immunology; History of public health; Poliomyelitis and polio

SACCHAROMYCES CEREVISIAE

Unicellular **Fungi** (**Yeast** Phylum) are one of the most studied single-cell **Eukaryotes**. Among them, *Saccharomyces cerevisiae* is perhaps the biological model most utilized for decades in order for scientists to understand the molecular anatomy and physiology of eukaryotic cells, such as membrane and transmembrane receptors, **cell cycle** controls, and **enzymes** and proteins involved in signal **transduction** to the **nucleus**.

Many strands of *S. cerevisiae* are used by the wine and beer industry for **fermentation**. *S. cerevisiae* is a member of the group of budding yeasts that replicate (reproduce) through the formation of an outgrowth in the parental cell known as a bud. After nuclear division into two daughter nuclei, one nucleus migrates to the bud, which continues to grow until it breaks off to form an independent daughter cell. Most eukaryotic cells undergo symmetric cell division, resulting in two daughter cells with the same size. In budding yeast, however, cell division is asymmetric and produces at cell separation a large parental cell and a small daughter cell. Moreover, after separation, the parental cell starts the production of a new bud, whereas the daughter cell continues to grow into its mature size before producing its own bud. Cell cycle times are also different between parental and young daughter cells. Parental (or mother cells) have a cell cycle of 100 minutes, whereas daughter cells in the growing process have a cycle time of 146 minutes from birth to first budding division.

The study of cell cycle controls, enzymatic systems of **DNA** repair, programmed cell death, and DNA **mutations** in *S. cerevisiae* and *S. pombe* greatly contributed to the understanding of pre-malignant cell transformations and the identification of genes involved in carcinogenesis. They constitute ideal biological models for these studies because they change the cellular shape in each phase of the cell cycle and in case of genetic mutation, the position defect is easily identified and related to the specific phase of the cell cycle. Such mutations are known as cdc mutations (cell division cycle mutations).

See also Cell cycle (eukaryotic), genetic regulation of; Yeast genetics

SALK, JONAS (1914-1995)

American physician

Jonas Salk was one of the United States's best-known microbiologists, chiefly celebrated for his discovery of his polio **vaccine**. Salk's greatest contribution to **immunology** was the insight that a "killed virus" is capable of serving as an **antigen**, prompting the body's **immune system** to produce antibodies that will attack invading organisms. This realization enabled Salk to develop a polio vaccine composed of killed polio **viruses**, producing the necessary antibodies to help the body to ward off the disease without itself inducing polio.

The eldest son of Orthodox Jewish-Polish immigrants, Jonas Edward Salk was born in East Harlem, New York, on October 28, 1914. His father, Daniel B. Salk, was a garment worker, who designed lace collars and cuffs and enjoyed

sketching in his spare time. He and his wife, Dora Press, encouraged their son's academic talents, sending him to Townsend Harris High School for the gifted. There, young Salk was both highly motivated and high achieving, graduating at the age of fifteen and enrolling in the legal faculty of the City College of New York. Ever curious, he attended some science courses and quickly decided to switch fields. Salk graduated with a bachelor's degree in science in 1933, at the age of nineteen, and went on to New York University's School of Medicine. Initially he scraped by on money his parents had borrowed for him; after the first year, however, scholarships and fellowships paid his way. In his senior year, Salk met the man with whom he would collaborate on some of the most important work of his career, Dr. Thomas Francis, Jr.

On June 7, 1939, Salk was awarded his M.D. The next day, he married Donna Lindsay, a psychology major who was employed as a social worker. The couple eventually had three sons. After graduation, Salk continued working with Francis, and concurrently began a two-year internship at Mount Sinai Hospital in New York. Upon completing his internship, Salk accepted a National Research Council fellowship and moved to The University of Michigan to join Dr. Francis, who had been heading up Michigan's department of **epidemiology** since the previous year. Working on behalf of the U.S. Army, the team strove to develop a flu vaccine. Their goal was a "killed-virus" vaccine—able to kill the live flu viruses in the body, while simultaneously producing antibodies that could fight off future invaders of the same type, thus producing **immunity**. By 1943, Salk and Francis had developed a formalin-killed-virus vaccine, effective against both type A and B **influenza** viruses, and were in a position to begin clinical trials.

In 1946, Salk was appointed assistant professor of epidemiology at Michigan. Around this time he extended his research to cover not only viruses and the body's reaction to them, but also their epidemic effects in populations. The following year he accepted an invitation to move to the University of Pittsburgh School of Medicine's Virus Research Laboratory as an associate research professor of bacteriology. When Salk arrived at the Pittsburgh laboratory, what he encountered was not encouraging. The laboratory had no experience with the kind of basic research he was accustomed to, and it took considerable effort on his part to bring the lab up to par. However, Salk was not shy about seeking financial support for the laboratory from outside benefactors, and soon his laboratory represented the cutting edge of viral research.

In addition to building a respectable laboratory, Salk also devoted a considerable amount of his energies to writing scientific papers on a number of topics, including the polio virus. Some of these came to the attention of Daniel Basil O'Connor, the director of the National Foundation for Infantile Paralysis—an organization that had long been involved with the treatment and rehabilitation of polio victims. O'Connor eyed Salk as a possible recruit for the polio vaccine research his organization sponsored. When the two finally met, O'Connor was much taken by Salk—so much so, in fact, that he put almost all of the National Foundation's money behind Salk's vaccine research efforts.

Poliomyelitis, traceable back to ancient Egypt, causes permanent paralysis in those it strikes, or chronic shortness of breath often leading to death. Children, in particular, are especially vulnerable to the polio virus. The University of Pittsburgh was one of four universities engaged in trying to sort and classify the more than one hundred known varieties of polio virus. By 1951, Salk was able to assert with certainty that all polio viruses fell into one of three types, each having various strains; some of these were highly infectious, others barely so. Once he had established this, Salk was in a position to start work on developing a vaccine.

Salk's first challenge was to obtain enough of the virus to be able to develop a vaccine in doses large enough to have an impact; this was particularly difficult since viruses, unlike culture-grown **bacteria**, need living cells to grow. The breakthrough came when the team of **John F. Enders**, **Thomas Weller**, and Frederick Robbins found that the polio virus could be grown in embryonic tissue—a discovery that earned them a Nobel Prize in 1954.

Salk subsequently grew samples of all three varieties of polio virus in cultures of monkey kidney tissue, then killed the virus with formaldehyde. Salk believed that it was essential to use a killed polio virus (rather than a live virus) in the vaccine, as the live-virus vaccine would have a much higher chance of accidentally inducing polio in inoculated children. He therefore, exposed the viruses to formaldehyde for nearly 13 days. Though after only three days he could detect no virulence in the sample, Salk wanted to establish a wide safety margin; after an additional ten days of exposure to the formaldehyde, he reasoned that there was only a one-in-a-trillion chance of there being a live virus particle in a single dose of his vaccine. Salk tested it on monkeys with positive results before proceeding to human clinical trials.

Despite Salk's confidence, many of his colleagues were skeptical, believing that a killed-virus vaccine could not possibly be effective. His dubious standing was further compounded by the fact that he was relatively new to polio vaccine research; some of his chief competitors in the race to develop the vaccine—most notably **Albert Sabin**, the chief proponent for a live-virus vaccine—had been at it for years.

As the field narrowed, the division between the killed-virus and the live-virus camps widened, and what had once been a polite difference of opinion became a serious ideological conflict. Salk and his chief backer, the National Foundation for Infantile Paralysis, were lonely in their corner. Salk failed to let his position in the scientific wilderness dissuade him and he continued, undeterred, with his research. To test his vaccine's strength, in early 1952, Salk administered a type I vaccine to children who had already been infected with the polio virus. Afterwards, he measured their **antibody** levels. His results clearly indicated that the vaccine produced large amounts of antibodies. Buoyed by this success, the clinical trial was then extended to include children who had never had polio.

In May 1952, Salk initiated preparations for a massive field trial in which over four hundred thousand children would be vaccinated. The largest medical experiment that had ever been carried out in the United States, the test finally got underway in April 1954, under the direction of Dr. Francis and spon-

sored by the National Foundation for Infantile Paralysis. More than one million children between the ages of six and nine took part in the trial, each receiving a button that proclaimed them a "Polio Pioneer." A third of the children were given doses of the vaccine consisting of three injections—one for each of the types of polio virus—plus a booster shot. A control group of the same number of children was given a placebo, and a third group was given nothing.

At the beginning of 1953, while the trial was still at an early stage, Salk's encouraging results were made public in the *Journal of the American Medical Association.* Predictably, media and public interest were intense. Anxious to avoid sensationalized versions of his work, Salk agreed to comment on the results thus far during a scheduled radio and press appearance.

Despite the doomsayers, on April 12, 1955, the vaccine was officially pronounced effective, potent, and safe in almost 90% of cases. The meeting at which the announcement was made was attended by five hundred of the world's top scientists and doctors, 150 journalists, and sixteen television and movie crews. The success of the trial catapulted Salk to instant stardom.

Wishing to escape from the glare of the limelight, Salk turned down the countless offers and tried to retreat into his laboratory. Unfortunately, a tragic mishap served to keep the attention of the world's media focused on him. Just two weeks after the announcement of the vaccine's discovery, eleven of the children who had received it developed polio; more cases soon followed. Altogether, about 200 children developed paralytic polio, eleven fatally. For a while, it appeared that the **vaccination** campaign would be railroaded. However, it was soon discovered that all of the rogue vaccines had originated from the same source, Cutter Laboratories in California. On May 7, the vaccination campaign was called to a halt by the Surgeon General. Following a thorough investigation, it was found that Cutter had used faulty batches of virus **culture**, which were resistant to the formaldehyde. After furious debate and the adoption of standards that would prevent such a reoccurrence, the inoculation resumed. By the end of 1955, seven million children had received their shots, and over the course of the next two years more than 200 million doses of Salk's polio vaccine were administered, without a single instance of vaccine-induced paralysis. By the summer of 1961, there had been a 96% reduction in the number of cases of polio in the United States, compared to the five-year period prior to the vaccination campaign.

After the initial inoculation period ended in 1958, Salk's killed-virus vaccine was replaced by a live-virus vaccine developed by Sabin; use of this new vaccine was advantageous because it could be administered orally rather than intravenously, and because it required fewer "booster" inoculations. To this day, though, Salk remains known as the man who defeated polio.

In 1954, Salk took up a new position as professor of preventative medicine at Pittsburgh, and in 1957 he became professor of experimental medicine. The following year he began work on a vaccine to immunize against all viral diseases of the central nervous system. As part of this research, Salk performed studies of normal and malignant cells, studies that had some bearing on the problems encountered in cancer research. In 1960, he founded the Salk Institute for Biological Studies in La Jolla, California; heavily funded by the National Foundation for Infantile Paralysis (by then known as the March of Dimes), the institute attracted some of the brightest scientists in the world, all drawn by Salk's promise of full-time, uninterrupted biological research.

Salk died on 23 June 1995, at a San Diego area hospital. His death, at the age of 80, was caused by heart failure.

See also Antibody and antigen; Antibody formation and kinetics; Immunity, active, passive and delayed; Immunization; Poliomyelitis and polio

SALMONELLA

Salmonella is the common name given to a type of food poisoning caused by the **bacteria** *Salmonella enteritidis* (other types of illnesses are caused by other species of *Salmonella* bacteria, including **typhoid fever**. When people eat food contaminated by *S. enteritidis,* they suffer **gastroenteritis** (**inflammation** of the stomach and intestines, with diarrhea and vomiting).

Salmonella food poisoning is most often caused by improperly handled or cooked poultry or eggs. Because chickens carrying the bacteria do not appear ill, infected chickens can lay eggs or be used as meat.

Early in the study of *Salmonella* food poisoning, it was thought that *Salmonella* bacteria were only found in eggs which had cracks in them, and that the infecting bacteria existed on the outside of the eggshell. Stringent guidelines were put into place to ensure that cracked eggs do not make it to the marketplace, and to make sure that the outside of eggshells were all carefully disinfected. However, outbreaks of *Salmonella* poisoning continued. Research then ultimately revealed that, because the egg shell has tiny pores, even uncracked eggs which have been left for a time on a surface (such as a chicken's roost) contaminated with *Salmonella* could become contaminated. Subsequently, further research has demonstrated that the bacteria can also be passed from the infected female chicken directly into the substance of the egg prior to the shell forming around it.

Currently, the majority of *Salmonella* food poisoning occurs due to unbroken, disinfected grade A eggs, which have become infected through bacteria which reside in the hen's ovaries. In the United States, he highest number of cases of *Salmonella* food poisoning occur in the Northeast, where it is believed that about one out of 10,000 eggs is infected with *Salmonella.*

The most effective way to avoid *Salmonella* poisoning is to properly cook all food which could potentially harbor the bacteria. Neither drying nor freezing are reliable ways to kill *Salmonella.* While the most common source for human infection with *Salmonella* bacteria is poultry products, other carriers include pets such as turtles, chicks, ducklings, and iguanas.

Products containing animal tissues may also be contaminated with *Salmonella*.

While anyone may contract *Salmonella* food poisoning from contaminated foods, the disease proves most threatening in infants, the elderly, and individuals with weakened immune systems. People who have had part or all of their stomach or spleen removed, as well as individuals with sickle cell anemia, cirrhosis of the liver, leukemia, lymphoma, **malaria**, louse-borne relapsing fever, or acquired **immunodeficiency** syndrome (**AIDS**) are particularly susceptible to *Salmonella* food poisoning. In the United States, about 15% of all cases of food poisoning are caused by *Salmonella*.

Salmonella food poisoning occurs most commonly when people eat undercooked chicken or eggs, sauces, salad dressings, or desserts containing raw eggs. The bacteria can also be spread if raw chicken, for example, contaminates a cutting board or a cook's hands, and is then spread to some other uncooked food. Cases of *Salmonella* infections in children have been traced to the children handling a pet (such as a turtle or an iguana) and then eating without first washing their hands. An individual who has had *Salmonella* food poisoning will continue to pass the bacteria into their feces for several weeks after the initial illness. Poor handwashing can allow others to become infected.

Symptoms of *Salmonella* food poisoning generally occur about 12–72 hours after ingestion of the bacteria. Half of all patients experience fever; other symptoms include nausea, vomiting, diarrhea, and abdominal cramping and pain. The stools are usually liquid, but rarely contain mucus or blood. Diarrhea usually lasts about four days. The entire illness usually resolves itself within about a week.

While serious complications of *Salmonella* food poisoning are rare, individuals with other medical illnesses are at higher risk. Complications occur when the *Salmonella* bacteria make their way into the bloodstream. Once in the bloodstream, the bacteria can invade any organ system, causing disease. Infections which can be caused by *Salmonella* include: bone infections (osteomyelitis), infections of the sac containing the heart (pericarditis), infections of the tissues which cover the brain and spinal cord (**meningitis**), and liver and lung infections.

Salmonella food poisoning is diagnosed by examining a stool sample. Under appropriate laboratory conditions, the bacteria in the stool can be encouraged to grow, and then processed and viewed under a **microscope** for identification.

Simple cases of *Salmonella* food poisoning are usually treated by encouraging good fluid intake, to avoid dehydration. Although the illness is caused by a bacteria, studies have shown that using **antibiotics** may not shorten the course of the illness. Instead, antibiotics may have the adverse effect of lengthening the amount of time the bacteria appear in the feces, thus potentially increasing others' risk of exposure to *Salmonella*. Additionally, some strains of *Salmonella* are developing resistance to several antibiotics.

Efforts to prevent *Salmonella* food poisoning have been greatly improved now that it is understood that eggs can be contaminated during their development inside the hen. Flocks are carefully tested, and eggs from infected chickens can be pasteurized to kill the bacteria. Efforts have been made to carefully educate the public about safe handling and cooking practices for both poultry and eggs. People who own pets that can carry *Salmonella* are also being more educated about more careful handwashing practices. It is unlikely that a human **immunization** will be developed, because there are so many different types of *Salmonella* enteritidis. However, researchers in 1997 produced an oral **vaccine** for poultry from genetically altered live *Salmonella* bacteria, currently undergoing testing, that may show the prevention of *Salmonella* bacteria from infecting meat or eggs. In 2001, two teams of researchers in England sequenced the genomes of both *Salmonella Typhimurium* (a common cause of food poisoning) and *Salmonella Typhi* the cause of typhoid fever). Data gathered from the project will improve diagnosis of *Salmonella* infections, and may eventually lead to a method of blocking its transmission in humans.

See also Antibiotic resistance, tests for; Bacteria and bacterial infection; Bacterial adaptation; Food safety

SALMONELLA FOOD POISONING

Salmonella food poisoning, consistent with all food poisoning, results from the growth of the bacterium in food. This is in contrast to food intoxication, were illness results from the presence of toxin in the food. While food intoxication does not require the growth of the contaminating **bacteria** to reasonably high numbers, food poisoning does.

Salmonella is a Gram negative, rod-shaped bacterium. The gastrointestinal tracts of man and animals are common sources of the bacterium. Often the bacterium is spread to food by handling the food with improperly washed hands. Thus, proper **hygiene** is one of the keys to preventing *Salmonella* food poisoning.

The food poisoning caused by *Salmonella* is one of about ten bacterial causes of food poisoning. Other involved bacteria are *Staphylococcus aureus*, *Clostridium perfringens*, *Vibrio parahaemolyticus*, and certain types of ***Escherichia coli***. Between 24 and 81 million cases of food borne diarrhea due to *Salmonella* and other bacteria occur in the United States each year. The economic cost of the illnesses is between 5 and 17 billion dollars.

Poultry, eggs, red meat, diary products, processed meats, cream-based desserts, and salad-type sandwich filling (such as tuna salad or chicken salad) are prime targets for colonization by species of *Salmonella*. The high protein content of the foodstuffs seems to be one of the reasons for their susceptibility. **Contamination** is especially facilitated if improperly cooked or raw food is held at an improper storage temperature, for example at room temperature. Proper cooking and storage temperatures will prevent contamination, as **Salmonella** is destroyed at cooking temperatures above 150° F (65.5 °C) and will not grow at refrigeration temperatures (less than 40°F, or 4.4°C). Also, contamination can result if the food is brought into contact with contaminated surfaces or utensils.

The vulnerable foods offer *Salmonella* a ready source of nutrients and moisture. If the temperature conditions are right for growth, the increase in numbers of *Salmonella* can be explosive. For example, from a starting population of a single live bacterium with a division time of 30 minutes, a population of over 500 million bacteria can be generated in just 15 hours.

The ingestion of contaminated foods leads, within hours, to the development of one or all of the following ailments: stomach cramps, vomiting, fever, headache, chills, sweating, fatigue, loss of appetite, and watery or bloody diarrhea. Prolonged diarrhea is dangerous, as the body can be depleted of fluids and salts that are vital for the proper functioning of organs and tissues. The resulting shock to the body can be intolerably lethal to infants and the elderly. As well, there is a possibility that the bacteria can spread from the intestinal tract to the bloodstream, leading to infections in other parts of the body.

There are hundreds are different forms, or strains, of *Salmonella,* varying in the antigenic composition of their outer surface and in the maladies caused. Concerning food poisoning, *Salmonella enteriditis* is of particular concern. This strain causes **gastroenteritis** and other maladies because of several so-called virulence factors the organism is armed with.

One virulence factor is called adhesin. An adhesin is a molecule that functions in the recognition and adhesion of the bacterium to a receptor on the surface of a host cell. In the case of *Salmonella,* the tube-like structures called fimbriae can perform this function. Other molecules on the surface of the bacterium can be involved also.

Another virulence factor is a compound called lipopolysaccharide (LPS for short). Depending on the structure, LPS can help shield the *Salmonella* surface from host antibacterial compounds. As well, part of the LPS, can lipid A, can be toxic to the host. The lipid A toxic component is also referred to as endotoxin. Salmonella also produces another toxin called **enterotoxin**. Other bacteria produce enterotoxin as well. The *Salmonella* enterotoxin is readily degraded by heat, so proper cooking of food will destroy the activity of the toxin. The enterotoxin remains inside the bacteria, so the toxin concentration increases with the increase in bacterial numbers.

Salmonella is not particularly difficult to identify, as it produces distinctive visual reactions on standard laboratory growth media. For example, on bismuth sulfide media the bacteria produce hydrogen sulfide, which produces jet-black colonies. Unfortunately for the individual who experiences a food poisoning event, the diagnosis is always "after the fact." Knowledge of the cause often comes after the miseries of the poisoning have come and gone. But, in those instances where the spread of the bacteria beyond the gastrointestinal tract has occurred, diagnosis is helpful to treat the infection.

The prospects of eliminating of *Salmonella* food poisoning using **vaccination** are being explored. The most promising route is to block the adhesion of the bacteria to host epithelial cells of the intestinal tract. Such a strategy would require the development of a **vaccine** with long lasting **immunity**. However, vaccine development efforts will likely be devoted to other illnesses. For the foreseeable future, the best strategy in preventing *Salmonella* food poisoning will remain the proper cooking of foods and the observance of good hygiene practices when handling food.

See also Food preservation

SCANNING ELECTRON MICROSCOPE • *see*

ELECTRON MICROSCOPE, TRANSMISSION AND SCANNING

SCHICK, BELA (1877-1967)

Hungarian-born American physician

Bela Schick was a pioneer in the field of child care; not only did he invent the **diphtheria** test, which helped wipe out this disease in children, but he also formulated and publicized child care theories that were advanced for his day. Schick also defined the allergic reaction, was considered the leading pediatrician of his time, and made contributions to knowledge about scarlet fever, **tuberculosis**, and infant nutrition. Schick received many honors for his work, including the Medal of the New York Academy of Medicine and the Addingham Gold Medal, a British award. Schick was also the founder of the American Academy of Pediatrics.

Schick was born on July 16, 1877 in Boglar, Hungary, the child of Jacob Schick, a grain merchant, and Johanna Pichler Schick. He attended the Staats Gymnasium in Graz, Austria, graduating in 1894. He then received his M.D. degree at Karl Franz University, also in Graz. After a stint with the medical corps in the Austro-Hungarian army, Schick started his own medical practice in Vienna in 1902. From then on he devoted his ample energies to teaching, research, and medical practice at the University of Vienna, where he served from 1902 to 1923—first as an intern, then as an assistant in the pediatrics clinic, and finally as lecturer and professor of pediatrics.

It was in 1905 that Schick made one of his most significant contributions. While working with collaborator Clemens von Pirquet, Schick wrote his first research study describing the phenomenon of allergy, which was then called serum sickness. The study not only described the concept of allergy, but also recommended methods of treatment.

At age 36, Schick moved on to make one of the most important discoveries of the twentieth century—the test for diphtheria. The test, announced in 1913, was a remarkably simple one that could tell whether a person was vulnerable to the disease. It showed whether a patient had already been exposed to the diphtheria toxin, which would make him immune from getting it again. A tiny amount of the diluted toxin was injected into the patient's arm. If the spot turned red and swollen, the doctors would know whether or not the patient been exposed to the disease. The treatment was then injection with an antitoxin.

Diphtheria was a common disease in the early twentieth century and afflicted thousands of children in every city throughout the world. It was especially common in Europe, where the close quarters of many cities made infection more

likely. At the time Schick embarked on his research, scientists had already isolated the microbe or toxin that caused diphtheria. A horse serum had also been developed that could prevent or even cure the disease. But the serum had so many side effects that doctors were unwilling to prescribe it unless they knew a patient was seriously in danger of catching diphtheria. Thus, Schick's discovery made it easier for them to treat those who were the most vulnerable.

In 1923, an antitoxin without side effects was developed and was then given to babies during their first year of life. Later on, the Schick test would show whether **immunity** persisted. Schick's test technique was also used years later to treat people with **allergies**, using the same technique of injecting small doses of an antitoxin.

Schick left Vienna in 1923 to become pediatrician-in-chief at Mt. Sinai Hospital in New York City. Schick became an American citizen that same year and two years later married his wife, Catherine C. Fries. He held his post at Mt. Sinai Hospital until his retirement in May 1943, when he became a consulting pediatrician. During his career, he also worked simultaneously at other hospitals, acting as director of pediatrics at Sea View Hospital in Staten Island, New York and consulting pediatrician at the Willard Parker Hospital, the New York Infirmary for Women and Children, and Beth Israel Hospital. He also taught as a professor of the diseases of children at Columbia University College of Physicians and Surgeons, starting in 1936.

Schick directed a private practice in New York City as well. His office held a collection of dolls and animals that he had acquired in travels throughout the world. He would often play the piano in his office, or take out one of his doll or animal figures to calm a child. He never displayed a stethoscope until he made sure a child was relaxed. At one time, he estimated that he had treated over a million children.

Childless himself, he had a great fondness for children and in 1932 authored a popular book titled *Child Care Today* that contained his firm beliefs about how children should be raised. Many of his ideas were advanced for his time. He advocated little punishment for children and no corporal punishment. He also said that trauma in a child's early life often had a lasting effect.

Schick and his wife lived in a large apartment in New York City and were frequent travelers around the world. On a cruise to South America with his wife during his later years, Schick fell ill with pleurisy. Eventually brought back to the United States to Mt. Sinai Hospital, he died on Dec. 6, 1967.

See also Allergies; History of immunology; History of microbiology; History of public health; Immune system; Immunology; Medical training and careers in microbiology

SCID

• *see* SEVERE COMBINED IMMUNODEFICIENCY (SCID)

SECONDARY IMMUNE RESPONSE

• *see* IMMUNITY, ACTIVE, PASSIVE, AND DELAYED

SELECTION

Evolutionary selection pressures act on all living organisms, regardless whether they are prokaryotic or higher **eukaryotes**. Selection refers to an evolutionary pressure that is the result of a combination of environmental and genetic pressures that affect the ability of an organism to live and, equally importantly, to produce reproductively successful offspring (including prokaryotic strains of cells).

As implied, natural selection involves the natural (but often complex) pressures present in an organism's environment. Artificial selection is the conscious manipulation of mating, manipulation, and fusion of genetic material to produce a desired result.

Evolution requires genetic variation, and these variations or changes (**mutations**) are usually deleterious because environmental factors already support the extent genetic distribution within a population.

Natural selection is based upon expressed differences in the ability of organisms to thrive and produce biologically successful offspring. Importantly, selection can only act to exert influence (drive) on those differences in **genotype** that appear as phenotypic differences. In a very real sense, evolutionary pressures act blindly.

There are three basic types of natural selection: directional selection favoring an extreme **phenotype**; stabilizing selection favoring a **phenotype** with characteristics intermediate to an extreme phenotype (i.e., normalizing selection); and disruptive selection that favors extreme phenotypes over intermediate genotypes.

The evolution of pesticide resistance provides a vivid example of directional selection, wherein the selective agent (in this case DDT) creates an apparent force in one direction, producing a corresponding change (improved resistance) in the affected organisms. Directional selection is also evident in the efforts of human beings to produce desired traits in many organisms ranging from **bacteria** to plants and animals.

Not all selective effects are directional, however. Selection can also produce results that are stabilizing or disruptive. Stabilizing selection occurs when significant changes in the traits of organisms are selected against. An example of this is birth weight in humans. Babies that are much heavier or lighter than average do not survive as well as those that are nearer the mean (average) weight.

On the other hand, selection is said to be disruptive if the extremes of some trait become favored over the intermediate values. Although not a factor for **microorganisms**, sexual selection and sexual dimorphism can influence the immunologic traits and capacity of a population.

Sometimes the fitness of a phenotype in some environment depends on how common (or rare) it is; this is known as frequency-dependent selection. Perhaps an animal enjoys an increased advantage if it conforms to the majority phenotype in the population. Conversely, a phenotype could be favored if it is rare, and its alternatives are in the majority. Frequency-dependent selection provides an interesting case in which the **gene** frequency itself alters the selective environment in which the genotype exists.

Many people attribute the phrase "survival of the fittest" to Darwin, but in fact, it originated from another naturalist/philosopher, Herbert Spencer (1820–1903). Recently, many recent evolutionary biologists have asked: Survival of the fittest what? At what organismal level is selection most powerful? What is the biological unit of natural selection-the species, the individual, or even the gene?

Selection can provide interesting consequences for bacteria and **viruses**. For example, reduced virulence in **parasites**, who depend on the survival of their hosts for their own survival may increase the reproductive success of the invading parasite. The *myxoma* virus, introduced in Australia to control imported European rabbits (*Oryctolagus cuniculus*), at first caused the deaths of many individuals. However, within a few years, the mortality rate was much lower, partly because the rabbits became resistant to the pathogen, but also partly because the virus had evolved a lower virulence. The reduction in the virulence is thought to have been aided because the virus is transmitted by a mosquito, from one living rabbit to another. The less deadly viral strain is maintained in the rabbit host population because rabbits afflicted with the more virulent strain would die before passing on the virus. Thus, the viral genes for reduced virulence could spread by group selection. Of course, reduced virulence is also in the interest of every individual virus, if it is to persist in its host. Scientists argue that one would not expect to observe evolution by group selection when individual selection is acting strongly in an opposing direction.

Some biologists, most notably Richard Dawkins (1941–), have argued that the gene itself is the true unit of selection. If one genetic alternative, or allele, provides its bearer with an adaptive advantage over some other individual who carries a different allele then the more beneficial allele will be replicated more times, as its bearer enjoys greater fitness. In his book *The Selfish Gene*, Dawkins argues that genes help to build the bodies that aid in their transmission; individual organisms are merely the "survival machines" that genes require to make more copies of themselves.

This argument has been criticized because natural selection cannot "see" the individual genes that reside in an organism's genome, but rather selects among phenotypes, the outward manifestation of all the genes that organisms possess. Some genetic combinations may confer very high fitness, but they may reside with genes having negative effects in the same individual. When an individual reproduces, its "bad" genes are replicated along with its "good" genes; if it fails to do so, even its most advantageous genes will not be transmitted into the next generation. Although the focus among most evolutionary biologists has been on selection at the level of the individual, this example raises the possibility that individual genes in genomes are under a kind of group selection. The success of single genes in being transmitted to subsequent generations will depend on their functioning well together, collectively building the best possible organism in a given environment.

When selective change is brought about by human effort, it is known as artificial selection. By allowing only a selected minority of individuals or specimen to reproduce, breeders can produce new generations of organisms (e.g. a particular virus or bacterium) that feature desired traits.

See also Epidemiology; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses; Rare genotype advantage

SELECTIVE MEDIA • *see* GROWTH AND GROWTH MEDIA

SEM • *see* ELECTRON MICROSCOPE, TRANSMISSION AND SCANNING

SEMMELWEIS, IGNAZ PHILIPP (1818-1865)

Hungarian physician

Along with American physician Oliver Wendell Holmes (1809–1894), Ignaz Semmelweis was one of the first two doctors worldwide to recognize the contagious nature of puerperal fever and promote steps to eliminate it, thereby dramatically reducing maternal deaths.

Semmelweis was born in Ofen, or Tabàn, then near Buda, now part of Budapest, Hungary, on July 1, 1818, the son of a Roman Catholic shopkeeper of German descent. After graduating from the Catholic Gymnasium of Buda in 1835 and the University of Pest in 1837, he went to the University of Vienna to study law, but immediately switched to medicine. He studied at Vienna until 1839, then again at Pest until 1841, then again at Vienna, earning his M.D. in 1844. Among his teachers were Karl von Rokitansky (1804–1878), Josef Skoda (1805–1881), and Ferdinand von Hebra (1816–1880). He did postgraduate work in Vienna hospitals in obstetrics, surgery, and, under Skoda, diagnostic methods. In 1846, he became assistant physician, tantamount to senior resident, at the obstetrical clinic of the Vienna General Hospital.

In the mid-nineteenth century, the maternal death rate for hospital births attended by physicians was much higher than for either home births or births attended by midwives. The principal killer was puerperal fever, or childbed fever, whose etiology was then unknown, but which **Louis Pasteur** (1822–1895) learned in 1879 was caused by a streptococcal infection of the open wound at the site of the placenta in women who had recently given birth. The infection could remain topical or it could pass through the uterus into the bloodstream and quickly become fatal. Before Semmelweis and Holmes, physicians generally assumed that puerperal fever was an unpreventable and natural consequence of some childbirths, and accepted the terrifying mortality statistics.

Witnessing so many healthy young mothers sicken and die greatly affected Semmelweis, and he grew determined to discover the cause and prevention of puerperal fever. Using Rokitanksy's pathological methods, he began a comparative study of autopsies of puerperal fever victims. The break-

through came when his fellow physician, Jakob Kolletschka (1803–1847), died of blood poisoning after cutting his finger while performing an autopsy. Semmelweis noticed that the pathological features of the autopsy on Kolletschka's body matched those of the autopsies of the puerperal fever victims. Semmelweis then only suspected, and did not prove, that the fever was a septicemia, an intrusion of **microorganisms** from a local infection into the bloodstream, but he instantly took action. In May 1847, he ordered all personnel under his authority to wash their hands between patients. This was a novel, radical, and unpopular rule, but in just a month the maternal death rate at the Vienna General Hospital dropped from twelve to two percent.

Even though Semmelweis had solid results and statistics on his side, many physicians simply refused to believe that washing their hands, which they considered undignified, could save lives. Resistance to his rule stiffened. Semmelweis made many powerful enemies, and in March 1849, he was demoted from his supervisory role. He served at St. Rochus Hospital in Pest from 1851 to 1857, but never achieved his former professional status.

Holmes was facing a similar crisis in America. In 1843, Holmes first claimed in print that puerperal fever was contagious. Semmelweis first published his findings in 1848. Now having heard of Semmelweis, Holmes in 1855, expanded his original article into a small book that explicitly praised Semmelweis. Likewise, having now heard of Holmes, Semmelweis published *Die Aetiologie, der Begriff, und die Prophylaxis des Kindbettfiebers* [The Etiology, Concept, and Prophylaxis of Childbed Fever] in 1861. The book was not well received. Semmelweis was a poor prose stylist, and his lack of writing skill adversely affected his campaign. Holmes, on the other hand, an accomplished essayist and poet as well as a first-rate physician, proved more persuasive, although it would still be thirty years before sanitary and hygienic methods became standard in American and European hospitals.

While no one ridiculed Holmes, who had enough charm and grace to forestall such attacks, Semmelweis became subject of mockery in the central European medical community. In 1863, the frustration he had long felt finally took its toll on his spirit. He became chronically depressed, unpredictably angry, socially withdrawn, and increasingly bitter. In July 1865, a coalition of colleagues, friends, and relatives committed him to the Niederösterreichische Heil-und Pflegeanstalt, an insane asylum in Döbling, near Vienna. He died there a month later, on August 13, 1865, from bacteremia due to an infected cut on his finger, with symptoms markedly akin to those of puerperal fever.

See also Bacteria and bacterial infection; Contamination, bacterial and viral; Germ theory of disease; Hygiene; Infection control; Streptococci and streptococcal infections; Transmission of pathogens; Viruses and responses to viral infection

SERILITY • *see* REPRODUCTIVE IMMUNOLOGY

SEROCONVERSION

Seroconversion is a term that refers to the development in the blood of antibodies to an infectious organism or agent. Typically, seroconversion is associated with infections caused by **bacteria**, **viruses**, and protozoans. But seroconversion also occurs after the deliberate inoculation with an **antigen** in the process of **vaccination**. In the case of infections, the development of detectable levels of antibodies can occur quickly, in the case of an active infection, or can be prolonged, in the case of a latent infection. Seroconversion typically heralds the development of the symptoms of the particular infection.

The phenomenon of seroconversion can be important in diagnosing infections that are caused by latent viruses. Examples of viruses include **hepatitis** B and C viruses, the Epstein Barr virus, and the **Human Immunodeficiency Virus** (**HIV**). When these viruses first infect people, the viral nucleic acid can become incorporated into the genome of the host. As a result, there will not be an immune response mounted against the virus. However, once viral replication has commenced antibodies to viral proteins can accumulate to detectable levels in the serum.

Seroconversion is am important aspect of Acquired **Immunodeficiency** Syndrome (**AIDS**). Antibodies to HIV can sometimes be detected shortly after infection with the virus, and before the virus becomes latent. Symptoms of infection at this stage are similar to the flu, and disappear quickly, so treatment is often not sought. If, however, diagnosis is made at this stage, based on presence of HIV antibodies, then treatment can begin immediately. This can be important to the future outlook of the patient, because often at this stage of the infection the **immune system** is relatively undamaged. If seroconversion occurs following activation of the latent virus, then immune destruction may already be advanced.

The presence of antibodies in the serum occurs much earlier in the case of infections that occur very soon after the introduction of the infectious microorganism. The type of **antibody** present can be used in the diagnosis of the infection. Additionally, seroconversion in the presence of symptoms but in the absence of detectable **microorganisms** (particularly bacteria) can be a hallmark of a chronic infection caused by the adherent bacterial populations known as biofilms. Again, the nature of the antibodies can help alert a physician to the presence of a hitherto undetected **bacterial infection**, and treatment can be started.

See also Antibiotic resistance, tests for; Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Immunity, active, passive and delayed; Immunochemistry; Immunodeficiency disease syndromes; Serology

SEROLOGY

Serology is the study of antigen-antibody reactions outside of a living organism (i.e., *in vitro*, in a laboratory setting). The

basis of serology is the recognition of an **antigen** by immune mechanisms, with the subsequent production of an **antibody**.

In medical terminology, serology refers to a blood test to detect the presence of antibodies against a microorganism. The detection of antibodies can be qualitative (i.e., determining whether the antibodies are present) or quantitative (i.e., determining the quantity of an antibody produced). Some **microorganisms** can stimulate the production of antibodies that persist in a person's blood for a long time. Thus, in a qualitative assay the detection of a particular antibody does not mean that the person has a current infection. However, it does mean that it is likely that at some time that person was infected with the particular microbial pathogen. Serology assays can be performed at various times and the level of antibody determined. If the antibody level rises, it usually is indicative of a response to an infection. The body produces elevated amounts of the antibody to help fight the challenging antigen.

Serology as a science began in 1901. Austrian American immunologist **Karl Landsteiner** (1868–1943) identified groups of red blood cells as A, B, and O. From that discovery came the recognition that cells of all types, including blood cells, cells of the body, and microorganisms carry proteins and other molecules on their surface that are recognized by cells of the **immune system**. There can be many different antigens on the surface of a microorganism, with many different antibodies being produced.

When the antigen and the antibody are in suspension together, they react together. The reaction can be a visible one, such as the formation of a precipitate made up of a complex of the antigen and the antibody. Other serology techniques are agglutination, complement-fixation and the detection of an antigen by the use of antibodies that have been complexed with a fluorescent compound.

Serological techniques are used in basic research, for example, to decipher the response of immune systems and to detect the presence of a specific target molecule. In the clinical setting, serology is used to confirm infections and to type the blood from a patient. Serology has also proven to be very useful in the area of forensics, where blood typing can be vital to establishing the guilt or innocence of a suspect, or the identity of a victim.

See also Antibody and antigen; Antibody formation and kinetics; Antibody-antigen, biochemical and molecular reactions; Bacteria and bacterial infection; Immune system; Laboratory techniques in immunology

SESSILE BACTERIA • *see* BIOFILM FORMATION AND DYNAMIC BEHAVIOR

SEVERE COMBINED IMMUNODEFICIENCY (SCID)

Severe combined **immunodeficiency** (SCID) is a rare genetic disease that is actually a group of inherited disorders characterized by a lack of immune response, usually occurring in infants less than six months old. SCID is the result of a combination of defects of both **T-lymphocytes** and B-lymphocytes. Lymphocytes are white blood cells that are made in bone marrow, and many move to the thymus gland where they become specialized immune T and **B cells**. In healthy individuals, **T cells** attack antigens while B cells make plasma cells that produce antibodies (**immunoglobulins**). However, this immune response in SCID patients is absent making them very susceptible to invading diseases, and thus children with untreated SCID rarely live to the age of two years.

SCID is characterized by three main features. The helper T-lymphocytes are functioning poorly or are absent, the thymus gland may be small and functioning poorly or is absent, and the stem cells in bone marrow, from which mature T- and B-lymphocytes arise, are absent or defective in their function. In all of these situations, little or no antibodies are produced. If, for example, T-lymphocytes are never fully developed, then the **immune system** can never function normally. Moreover, the results of these defects include the following: impairment of normal functioning T- and B-lymphocytes, negative effects on the maturation process for T-helper and T-suppressor cells, and elimination and damage of the original source of the lymphocytes.

The immune disorders characterized in SCID arise because of the inheritance of abnormal genes from one or both parents. The most common form of SCID is linked to the X chromosome inherited from the mother; this makes SCID more common among males. The second most common defect is caused by the inheritance of both parents' abnormally inactive genes governing the production of a particular enzyme that is needed for the development of **immunity**, called adenosine deaminase (ADA). Although many defective genes for other forms of SCID have been identified in the last few years, scientists do not fully understand all of the forms of the disease.

There are many specific clinical signs that are associated with SCID. After birth, an infant with SCID is initially protected by the temporarily active maternal immune cells; however, as the child ages, his or her immune system fails to take over as the maternal cells become inactive. Pulmonary problems such as **pneumonia**, non-productive coughs, **inflammation** around the bronchial tubes, and low alveolar oxygen levels can affect the diseased infant repetitively. Chronic diarrhea is not uncommon, and can lead to severe weight loss, malnutrition, and other gastrointestinal problem. Infants with the disease have an unusual number of bacterial, fungal, viral, or protozoal infections that are much more resistant to treatment than in healthy children. Mouth **thrush** and **yeast** infections, both fungal, appear in SCID patients and are very resistant to treatment. Additionally, chronic bacterial and fungal **skin infections** and several abnormalities of the blood cells can persist.

Severe combined immunodeficiency is a disease that can be successfully treated if it is identified early. The most effective treatment has been hematopoietic stem cell transplants that are best done with the bone marrow of a sister or brother; however, the parent's marrow is acceptable if the infant is less than three months old. Early treatment can also

help to avoid pre-transplant **chemotherapy** often necessary to prevent rejection of the marrow in older children. This is especially advantageous because chemotherapy can leave the patient even more susceptible to invading bodies. When successful, treatment for SCID corrects the patient's immune system defect, and as of 2002 success rates have been shown to be nearly 80% for the bone marrow transplant.

Gene therapy is the subject of ongoing research, and shows promise as a treatment for SCID. Researchers remove T cells of SCID patients and expose those cells to the ADA gene for ten days, and then return the cells intravenously. Although it was successful in one case, this treatment of SCID is still very much in the experimental stage. Nevertheless, these and other treatments hold potential for the development of a cure for SCID.

See also Immune system; Immunochemistry; Immunodeficiency disease syndromes; Immunodeficiency diseases; Immunogenetics; Immunoglobulins and immunoglobulin deficiency syndromes; Immunological analysis techniques; Immunology

SEXUALLY TRANSMITTED DISEASES (STDS)

Sexually transmitted diseases (STDs) vary in their susceptibility to treatment, their signs and symptoms, and the consequences if they are left untreated. Some are caused by **bacteria**. These usually can be treated and cured. Others are caused by **viruses** and can typically be treated but not cured. As of June 2002, recent advancements in diagnosis now allow the identification of more than 15 million new cases of STD in the United States each year.

Long known as venereal disease, after Venus, the Roman goddess of love, sexually transmitted diseases are increasingly common. The more than 20 known sexually transmitted diseases range from the life-threatening to painful and unsightly. The life-threatening sexually transmitted diseases include **syphilis**, which has been known for centuries, some forms of **hepatitis**, and Acquired Immune Deficiency Syndrome (**AIDS**), which was first identified in 1981.

Most sexually transmitted diseases can be treated successfully, although untreated sexually transmitted diseases remain a huge **public health** problem. Untreated sexually transmitted diseases can cause everything from blindness to infertility. While AIDS is the most widely publicized sexually transmitted disease, others are more common. More than 13 million Americans of all backgrounds and economic levels develop sexually transmitted diseases every year. Prevention efforts focus on teaching the physical signs of sexually transmitted diseases, instructing individuals on how to avoid exposure, and emphasizing the need for regular check-ups.

The history of sexually transmitted disease is controversial. Some historians argue that syphilis emerged as a new disease in the fifteenth century. Others cite Biblical and other ancient texts as proof that syphilis and perhaps **gonorrhea** were ancient as well as contemporary burdens. The dispute can best be understood with some knowledge of the elusive nature of gonorrhea and syphilis, called "the great imitator" by the eminent physician William Osler (1849–1919).

No laboratory tests existed to diagnose gonorrhea and syphilis until the late nineteenth and early twentieth centuries. This means that early clinicians based their diagnosis exclusively on symptoms, all of which could be present in other illnesses. Symptoms of syphilis during the first two of its three stages include chancre sores, skin rash, fever, fatigue, headache, sore throat, and swollen glands. Likewise, many other diseases have the potential to cause the dire consequences of late-stage syphilis. These range from blindness to mental illness to heart disease to death. Diagnosis of syphilis before laboratory tests were developed was complicated by the fact that most symptoms disappear during the third stage of the disease.

Symptoms of gonorrhea may also be elusive, particularly in women. Men have the most obvious symptoms, with **inflammation** and discharge from the penis from two to ten days after infection. Symptoms in women include a painful sensation while urinating or abdominal pain. However, women may be infected for months without showing any symptoms. Untreated gonorrhea can cause infertility in women and blindness in infants born to women with the disease.

The nonspecific nature of many symptoms linked to syphilis and gonorrhea means that historical references to sexually transmitted disease are open to different interpretations. There is also evidence that sexually transmitted disease was present in ancient China.

During the Renaissance, syphilis became a common and deadly disease in Europe. It is unclear whether new, more dangerous strains of syphilis were introduced or whether the syphilis which emerged at that time was, indeed, a new illness. Historians have proposed many arguments to explain the dramatic increase in syphilis during the era. One argument suggests that Columbus and other explorers of the New World carried syphilis back to Europe. In 1539, the Spanish physician Rodrigo Ruiz Diaz de Isla treated members of the crew of Columbus for a peculiar disease marked by eruptions on the skin. Other contemporary accounts tell of **epidemics** of syphilis across Europe in 1495.

The abundance of syphilis during the Renaissance made the disease a central element of the dynamic **culture** of the period. The poet John Donne (1572-1631) was one of many thinkers of that era who saw sexually transmitted disease as a consequence of man's weakness. Shakespeare (1564-1616) also wrote about syphilis, using it as a curse in some plays and referring to the "tub of infamy," a nickname for a common medical treatment for syphilis. The treatment involved placing syphilitic individuals in a tub where they received mercury rubs. Mercury, which is now known to be a toxic chemical, did not cure syphilis, but is thought to have helped relieve some symptoms. Other treatments for syphilis included the induction of fever and the use of purgatives to flush the system.

The sculptor Benvenuto Cellini (1500–1571) is one of many individuals who wrote about their own syphilis during the era: "The French disease, for it was that, remained in me

more than four months dormant before it showed itself." Cellini's reference to syphilis as the "French disease" was typical of Italians at the time and reflects a worldwide eagerness to place the origin of syphilis far away from one's own home. The French, for their part, called it the "Neapolitan disease," and the Japanese called it the "Portuguese disease." The name syphilis was bestowed on the disease by the Italian Girolamo Fracastoro (1478–1553), a poet, physician, and scientist. Fracastoro created an allegorical story about syphilis in 1530 entitled "Syphilis, or the French Disease." The story proposed that syphilis developed on Earth after a shepherd named Syphilis foolishly cursed at the Sun. The angry Sun retaliated with a disease that took its name from the foolish shepherd, who was the first individual to get sick.

For years, medical experts used syphilis as a catch-all diagnosis for sexually transmitted disease. Physicians assumed that syphilis and gonorrhea were the same thing until 1837, when Philippe Ricord (1800–1889) reported that syphilis and gonorrhea were separate illnesses. The late nineteenth and early twentieth centuries saw major breakthroughs in the understanding of syphilis and gonorrhea. In 1879, Albert Neisser (1855–1916) discovered that gonorrhea was caused by a bacillus, which has since been named *Neisseria gonorrhoeae*. Fritz Richard Schaudinn (1871–1906) and Paul Erich Hoffmann (1868–1959) identified a special type of spirochete bacteria, now known as Treponema pallidum, as the cause of syphilis in 1905.

Further advances occurred quickly. August von Wassermann (1866–1925) developed a blood test for syphilis in 1906, making testing for syphilis a simple procedure for the first time. Just four years later in 1910, the first effective therapy for syphilis was introduced in the form of Salvarsan, an organic arsenical compound. The compound was one of many effective compounds introduced by the German physician **Paul Ehrlich** (1854–1915), whose argument that specific drugs could be effective against **microorganisms** has proven correct. The drug is effective against syphilis, but it is toxic and even fatal to some patients.

The development of Salvarsan offered hope for individuals with syphilis, but there was little public understanding about how syphilis was transmitted in the early twentieth century. In the United States, this stemmed in part from government enforcement of laws prohibiting public discussion of certain types of sexual information. One popular account of syphilis from 1915 erroneously warned that one could develop syphilis after contact with whistles, pens, pencils, toilets, and toothbrushes.

In a tragic chapter in American history, some members of the U.S. Public Health Service exploited the ignorance of the disease among the general public as late as the mid-twentieth century in order to study the ravages of untreated syphilis. The Tuskegee Syphilis Study was launched in 1932 by the U.S. Public Health Service. The almost 400 black men who participated in the study were promised free medical care and burial money. Although effective treatments had been available for decades, researchers withheld treatment, even when **penicillin** became available in 1943, and carefully observed the unchecked progress of symptoms. Many of the participants fathered children with congenital syphilis, and many died. The study was finally exposed in the media in the early 1970s. When the activities of the study were revealed, a series of new regulations governing human experimentation were passed by the government.

A more public discussion of sexually transmitted disease was conducted by the military during World Wars I and II. During both wars, the military conducted aggressive public information campaigns to limit sexually transmitted disease among the armed forces. One poster from World War II showed a grinning skull on a woman dressed in an evening gown striding along with German Chancellor Führer Adolf Hitler and Japanese Emperor Hirohito. The poster's caption reads "V.D. Worst of the Three," suggesting that venereal disease could destroy American troops faster than either of America's declared enemies.

Concern about the human cost of sexually transmitted disease helped make the production of the new drug penicillin a wartime priority. Arthur Fleming (1881–1955), who is credited with the discovery of penicillin, first observed in 1928 that the penicillium **mold** was capable of killing bacteria in the laboratory; however, the mold was unstable and difficult to produce. Penicillin was not ready for general use or general clinical testing until after Howard Florey (1898–1968) and **Ernst Boris Chain** (1906–1979) developed ways to purify and produce a consistent substance.

The introduction of penicillin for widespread use in 1943 completed the **transformation** of syphilis from a life–threatening disease to one that could be treated relatively easily and quickly. United States rates of cure were 90–97% for syphilis by 1944, one year after penicillin was first distributed in the country. Death rates dropped dramatically. In 1940, 10.7 out of every 100,000 people died of syphilis. By 1970, it was 0.2 per 100,000.

Such progress infused the medical community with optimism. A 1951 article in the American Journal of Syphilis asked, "Are Venereal Diseases Disappearing?" By 1958, the number of cases of syphilis had dropped to 113,884 from 575,593 in 1943, the year penicillin was introduced.

Venereal disease was not eliminated, and sexually transmitted diseases continue to ravage Americans and others in the 1990s. Though penicillin has lived up to its early promise as an effective treatment for syphilis, the number of cases of syphilis has increased since 1956. In addition, millions of Americans suffer from other sexually transmitted diseases, many of which were not known a century or more ago, such as Acquired Immune Deficiency Syndrome (AIDS) caused by the **HIV** virus. By the 1990s, sexually transmitted diseases were among the most common infectious diseases in the United States.

Some sexually transmitted diseases are seen as growing at epidemic rates. For example, syphilis, gonorrhea, and chancroid, which are uncommon in Europe, Japan and Australia, have increased at epidemic rates among certain urban minority populations. A 1990 study found the rate of syphilis was more than four times higher among blacks than among whites. The Public Health Service reports that as many as 30 million Americans have been affected by genital **herpes**. Experts have

also noted that sexually transmitted disease appears to increase in areas where AIDS is common.

Shifting sexual and marital habits are two factors behind the growth in sexually transmitted disease. Americans are more likely to have sex at an earlier age than they did in years past. They also marry later in life than Americans did two to three decades ago, and their marriages are more likely to end in divorce. These factors make Americans more likely to have many sexual partners over the course of their lives, placing them at greater risk of sexually transmitted disease.

Public health officials report that fear and embarrassment continue to limit the number of people willing to report signs of sexually transmitted disease.

All sexually transmitted diseases have certain elements in common. They are most prevalent among teenagers and young adults, with nearly 66% occurring in people under 25. In addition, most can be transmitted in ways other than through sexual relations. For example, AIDS and Hepatitis B can be transmitted through contact with tainted blood, but they are primarily transmitted sexually. In general, sexual contact should be avoided if there are visible sores, warts, or other signs of disease in the genital area. The risk of developing most sexually transmitted diseases is reduced by using condoms and limiting sexual contact—but can only be reduced to zero by having monogamous (one partner) sexual relations between partners who are free of disease or vectors of disease (e.g., the HIV virus).

Bacterial sexually transmitted diseases include syphilis, gonorrhea, chlamydia, and chancroid. Syphilis is less common than many other sexually transmitted diseases in the Unites States, with 134,000 cases in 1990. The disease is thought to be more difficult to transmit than many other sexually transmitted diseases. Sexual partners of an individual with syphilis have about a 10% chance of developing syphilis after one sexual contact, but the disease has come under increasing scrutiny as researchers have realized how easily the HIV virus which causes AIDS can be spread through open syphilitic chancre sores.

Gonorrhea is far more common than syphilis, with approximately 750,000 cases of gonorrhea reported annually in the United States. The gonococcus bacterium is considered highly contagious. Public health officials suggest that all individuals with more than one sexual partner should be tested regularly for gonorrhea. Penicillin is no longer the treatment of choice for gonorrhea, because of the numerous strains of gonorrhea that are resistant to penicillin. Newer strains of **antibiotics** have proven to be more effective. Gonorrhea infection overall has diminished in the United States, but the incidence of gonorrhea among certain populations (e.g., African-Americans) has increased.

Chlamydia infection is considered the most common sexually transmitted disease in the United States. About four million new cases of chlamydia infection are reported every year. The infection is caused by the bacterium Chlamydia trachomatis. Symptoms of chlamydia are similar to symptoms of gonorrhea, and the disease often occurs at the same time as gonorrhea. Men and women may have pain during urination or notice an unusual genital discharge one to three weeks after exposure. However, many individuals, particularly women, have no symptoms until complications develop.

Complications resulting from untreated chlamydia occur when the bacteria has a chance to travel in the body. Chlamydia can result in pelvic inflammatory disease in women, a condition which occurs when the infection travels up the uterus and fallopian tubes. This condition can lead to infertility. In men, the infection can lead to epididymitis, inflammation of the epididymis, a structure on the testes where spermatozoa are stored. This too can lead to infertility. Untreated chlamydia infection can cause eye infection or **pneumonia** in babies of mothers with the infection. Antibiotics are successful against chlamydia.

The progression of chancroid in the United States is a modern-day indicator of the migration of sexually transmitted disease. Chancroid, a **bacterial infection** caused by *Haemophilus ducreyi*, was common in Africa and rare in the United States until the 1980s. Beginning in the mid-1980s, there were outbreaks of chancroid in a number of large cities and migrant-labor communities in the United States. The number of chancroid cases increased dramatically during the last two decades of the twentieth century.

In men, who are most likely to develop chancroid, the disease is characterized by painful open sores and swollen lymph nodes in the groin. The sores are generally softer than the harder chancre seen in syphilis. Women may also develop painful sores. They may feel pain urinating and may have bleeding or discharge in the rectal and vaginal areas. Chancroid can be treated effectively with antibiotics.

As of June 2002, there are no cures for the sexually transmitted diseases caused by viruses: AIDS, genital herpes, viral hepatitis, and genital warts. Treatment to reduce adverse symptoms is available for most of these diseases, but the virus cannot be eliminated from the body.

AIDS is the most life-threatening sexually transmitted disease, a disease which is usually fatal and for which there is no cure. The disease is caused by the **human immunodeficiency virus** (HIV), a virus which disables the **immune system**, making the body susceptible to injury or death from infection and certain cancers. HIV is a retrovirus which translates the **RNA** contained in the virus into **DNA**, the genetic information code contained in the human body. This DNA becomes a part of the human host cell. The fact that viruses become part of the human body makes them difficult to treat or eliminate without harming the patient.

HIV can remain dormant for years within the human body. More than 800,000 cases of AIDS have been reported in the United States **Centers for Disease Control** since the disease was first identified in 1981, and at least one million other Americans are believed to be infected with the HIV virus. Initial symptoms of AIDS include fever, headache, or enlarged lymph nodes. Later symptoms include energy loss, frequent fever, weight loss, or frequent **yeast** infections. HIV is transmitted most commonly through sexual contact or through use of contaminated needles or blood products. The disease is not spread through casual contact, such as the sharing of towels, bedding, swimming pools, or toilet seats.

Genital herpes is a widespread, recurrent, and incurable viral infection. Almost a million new cases are reported in the United States annually. The prevalence of herpes infection reflects the highly contagious nature of the virus. About 75% of the sexual partners of individuals with the infection develop genital herpes.

The herpes virus is common. Most individuals who are exposed to one of the two types of herpes simplex virus never develop any symptoms. In these cases, the herpes virus remains in certain nerve cells of the body, but does not cause any problems. Herpes simplex virus type 1 most frequently causes cold sores on the lips or mouth, but can also cause genital infections. Herpes simplex virus type 2 most commonly causes genital sores, though mouth sores can also occur due to this type of virus.

In genital herpes, the virus enters the skin or mucous membrane, travels to a group of nerves at the end of the spinal cord, and initiates a host of painful symptoms within about one week of exposure. These symptoms may include vaginal discharge, pain in the legs, and an itching or burning feeling. A few days later, sores appear at the infected area. Beginning as small red bumps, they can become open sores which eventually become crusted. These sores are typically painful and last an average of two weeks.

Following the initial outbreak, the virus waits in the nerve cells in an inactive state. A recurrence is created when the virus moves through the nervous system to the skin. There may be new sores or simply a shedding of virus which can infect a sexual partner. The number of times herpes recurs varies from individual to individual, ranging from several times a year to only once or twice in a lifetime. Occurrences of genital herpes may be shortened through use of an antiviral drug which limits the herpes virus's ability to reproduce itself.

Genital herpes is most dangerous to newborns born to pregnant women experiencing their first episode of the disease. Direct newborn contact with the virus increases the risk of neurological damage or death. To avoid exposure, physicians usually deliver babies using cesarean section if herpes lesions are present.

Hepatitis, an inflammation of the liver, is a complicated illness with many types. Millions of Americans develop hepatitis annually. The hepatitis A virus, one of four types of viral hepatitis, is most often spread by **contamination** of food or water. The hepatitis B virus is most often spread through sexual contact, through the sharing of intravenous drug needles, and from mother to child. Hospital workers who are exposed to blood and blood products are also at risk. Hepatitis C and Hepatitis D (less commonly) may also be spread through sexual contact.

A yellowing of the skin, or jaundice, is the best known symptom of hepatitis. Other symptoms include dark and foamy urine and abdominal pain. There is no cure for hepatitis, although prolonged rest usually enables individuals with the disease to recover completely.

Many people who develop hepatitis B become carriers of the virus for life. This means they can infect others and face a high risk of developing liver disease. There are as many as 350 million carriers worldwide, and about 1.5 million in the United States. A **vaccination** is available against hepatitis B.

The link between human papillomavirus, genital warts, and certain types of cancer has drawn attention to the potential risk of genital warts. There are more than 60 types of human papillomavirus. Many of these types can cause genital warts. In the United States, about 1 million new cases of genital warts are diagnosed every year.

Genital warts are very contagious, and about two-thirds of the individuals who have sexual contact with someone with genital warts develop the disease. There is also an association between human papillomavirus and cancer of the cervix, anus, penis, and vulva. This means that people who develop genital warts appear to be at a higher risk for these cancers and should have their health carefully watched. Contact with genital warts can also damage infants born to mothers with the problem.

Genital warts usually appear within three months of sexual contact. The warts can be removed in various ways, but the virus remains in the body. Once the warts are removed the chances of transmitting the disease are reduced.

Many questions persist concerning the control of sexually transmitted diseases. Experts have struggled for years with efforts to inform people about transmission and treatment of sexually transmitted disease. Frustration over the continuing increase in sexually transmitted disease is one factor which has fueled interest in potential vaccines against certain sexually transmitted diseases.

A worldwide research effort to develop a **vaccine** against AIDS has resulted in a series of vaccinations now in clinical trials. Efforts have focused in two areas, finding a vaccine to protect individuals against the HIV virus and finding a vaccine to prevent the progression of HIV to AIDS in individuals who already have been exposed to the virus. One of many challenges facing researchers has been the ability of the HIV virus to change, making efforts to develop a single vaccine against the virus futile.

Researchers also are searching for vaccines against syphilis and gonorrhea. Experiments conducted on prisoners more than 40 years ago proved that some individuals could develop **immunity** to syphilis after inoculation with live *Treponema pallidum*, but researchers have still not been able to develop a vaccine against syphilis which is safe and effective. In part this stems from the unusual nature of the syphilis bacteria, which remain potentially infectious even when its cells are killed. An effective gonorrhea vaccine has also eluded researchers.

Immunizations are available against Hepatitis A and Hepatitis B (Hepatitis D is prevented by the Hepatitis B vaccine). The virus that causes Hepatitis C, however, is able to change its form (mutate) quite rapidly, thereby hampering efforts to develop a vaccine against it.

Without vaccinations for most of the sexually transmitted diseases, health officials depend on public information campaigns to limit the growth of the diseases. Some critics have claimed that the increasing incidence of sexually transmitted diseases suggest that current techniques are failing. In other countries, however, the incidence of sexually transmitted disease has fallen during the same period it has risen in the

United States. For example, in Sweden the gonorrhea rate fell by more than 95% from 1970 to 1989 after vigorous government efforts to control sexually transmitted disease in Sweden.

Yet the role of government funding for community health clinics, birth control, and public information campaigns on sexually transmitted disease has long been controversial. Public officials continue to debate the wisdom of funding public distribution of condoms and other services that could affect the transmission of sexually transmitted disease. Although science has made great strides in understanding the causes and cures of many sexually transmitted diseases, society has yet to reach agreement on how best to attack them.

See also Bacteria and bacterial infection; Immunization; Immunogenetics; Public health, current issues; Virus replication; Viruses and responses to viral infection

SHEATHED BACTERIA

Sheathed **bacteria** are bacteria that grow as long filaments whose exterior is covered by a layer known as a sheath. Within the sheath, the bacteria can be capable of growth and division. Examples of sheathed bacteria include *Leptothrix discophora* (also known as "iron bacteria"), and *Sphaerotilus natans*.

Sheathed bacteria are common of the bacterial communities in water and in soil. In these environments, the sheath is often coated with precipitates of elements in the water or soil environments, such as oxides of iron and manganese. The elements are unstable in solution, and thus will readily come out of solution when presented with an appropriate site.

The sheath that covers the bacteria can be of varied construction. Much of the structural information has been gleaned from the observation of thin slices of sample using the transmission **electron microscope**. The sheath surrounding *Leptothrix* species is glycocalyx-like in appearance. Often the deposition of metals within the sheath network produces areas where the material has crystallized. In contrast, the sheath of *Sphaerotilus natans* presents the "railroad track" appearance, which is typical of a biological membrane consisting of two layers of lipid molecules.

Electron microscopic studies of *Leptothrix* species have shown that the bacterium is intimately connected with the overlying sheath. The connections consist of protuberances that are found all over the surface of the bacterium. In contrast, *Sphaerotilus natans* is not connected with the overlying sheath.

Both *Leptothrix* and *Sphaerotilus natans* can exist independently of the sheath. Bacteria in both genera have a life cycle that includes a free-swimming form (called a swarmer cell) that is not sheathed. The free-swimming forms have flagella at one end of the bacteria that propels the cells along. When encased in the sheath, the bacteria are referred to as sheathed or resting bacteria.

Bacterial sheaths tend to be manufactured when the bacteria are in an aquatic or soil environment that contains high amounts of organic matter. The sheath may serve to provide protection to the bacteria in these environments, Also, the ability of metallic compounds to precipitate on the sheath may provide the bacteria with a ready supply of such inorganic nutrients. For example, *Leptothrix* is able to utilize the manganese contained in the manganese oxide precipitate on the sheath.

Sheaths may also help the bacteria survive over a wide range of temperature and **pH**, by providing a relatively inert barrier to the external environment.

See also Bacterial appendages; Soil formation, involvement of microorganisms

SHIGELLA

Shigella is a genus of Gram-negative **bacteria** that is similar in behavior and habitat to ***Escherichia coli***. The bacterium is named after its discoverer, Japanese scientist Kiyoshi Shiga. The bacteria were discovered over 100 years ago.

Some strains of the bacteria can produce toxins, including the so-called Shiga toxin, which is very similar to the destructive verotoxin of *Escherichia coli* O157:H7. Indeed, strain O157:H7 is now presumed to have arisen by virtue of a genetic **recombination** between strains of *Shigella* and *Escherichia coli* in the intestinal tract, which resulted in the acquisition of the verotoxin by *Escherichia coli*.

The similarity between *Shigella* and *Escherichia coli* extends to the structure of the bacteria and their utilization of certain compounds as nutrients. The similarity is so pronounced that *Shigella* has been regarded as a strain of *Escherichia coli*. However, this is now known not to be the case. *Shigella* does not produce gas from the utilization of carbohydrates, while *Escherichia coli* does.

Shigella is one of a group of bacteria, which includes *Escherichia coli*, that inhabits the intestinal tract of humans and other warm blooded animals. Most strains of the bacterium are innocuous. However, the strains that possess the destructive toxins can do much damage to the intestinal wall and other areas of the body.

There are a number of *Shigella* species that are noteworthy to humans. *Shigella sonnei*, which is also known as group D *Shigella*, is the cause of almost 70 percent of the reported cases of food-borne *Shigella* illness in the United States each year. *Shigella flexneri*, which is also called group B *Shigella*, is responsible for virtually all the remaining cases of food-borne illness. In underdeveloped countries of the world, the bacterium *Shigella dysenteriae* type 1 is epidemic in its scope.

The illness that is caused by *Shigella* species is called shigellosis. The illness is classified as a bacillary **dysentery**. An estimated 300,000 cases of shigellosis occurs in the United States each year. Production of the toxins following the ingested of *Shigella*-contaminated food produces the illness. The illness is characterized by pain in the abdomen, cramps, diarrhea that can become bloody as intestinal cells are damaged, vomiting, and fever. These symptoms typically begin from 12 hours to three days after consuming food that is contaminated with the microorganism. **Contamination** usually results from the exposure of the food to feces-contaminated water or from improper **hygiene** prior to the handling of the

food. Both are routes of transfer of fecal material to the food. The amount of fecal material need not be great, as studies have proven that only 10 living *Shigella* are required to establish an infection in humans.

The infection tends to be fairly short in duration and clears without any therapeutic intervention. In some people, however, the primary infection can be the prelude to very damaging infections of the kidney and the joins. The latter infection, which is caused by *Shigella flexneri*, is known as Reiter's syndrome. This can persist for years. During this time, infections by other strains of *Shigella* are possible.

Shigellosis results from the attachment of the bacteria to epithelial cells that line the intestinal tract, and the entry of the bacteria into the cells. Within the host cells, the bacteria divide and can then spread laterally to infect other host cells. The interior location of the bacteria protects them from any host immune response or from **antibiotics**. Additionally, some strains of *Shigella* produce the toxins that can damage the epithelial cells.

The establishment of an infection is easier in people whose immune systems are compromised. For example, shigellosis is a significant problem in those afflicted with acquired **immunodeficiency** syndrome.

Treatment for *Shigella* infections is not always clinically prudent. Many infections, while very inconvenient and painful, pass relatively quickly. Management of the symptoms, particularly ensuring proper hydration, is preferred in immunocompetent people, as opposed to antibiotic therapy. The reason is that the bacteria can rather readily acquire resistance to antibiotics, which can make eradication of the bacteria even harder. Also, the antibiotic resistant bacteria can be excreted in the feces of the infected individual, and may then spread the resistant strain to other people.

Prevention of the spread of infection involves proper hygiene and thorough cooking of foods.

See also Enterobacteriaceae; Enterotoxin and exotoxin; Food safety

SHOTGUN CLONING

The shotgun method (also known as shotgun cloning) is a method in cloning genomic **DNA**. It involves taking the DNA to be cloned and cutting it either using a restriction enzyme or randomly using a physical method to smash the DNA into small pieces. These fragments are then taken together and cloned into a vector. The original DNA can be either genomic DNA (whole genome shotgun cloning) or a clone such as a **YAC** (**yeast artificial chromosome**) that contains a large piece of genomic DNA needing to be split into fragments.

If the DNA needs to be in a certain cloning vector, but the vector can only carry small amounts of DNA, then the shotgun method can be used. More commonly, the method is used to generate small fragments of DNA for sequencing. A DNA sequence can be generated at about 600 bases at a time, so if a DNA fragment of about 1100kb is cloned, then it can be sequenced in two steps, with 600 bases from each end, and a hundred base overlap. The sequencing can always be primed with a known sequence from the vector and so any prior knowledge of the sequence that has been cloned is not necessary. This approach of shotgun cloning followed by DNA sequencing from both ends of the vector is called shotgun sequencing.

Shotgun sequencing was initially used to sequence small genomes such as that of the cauliflower mosaic virus (CMV), which is 8kb long. More recently, it has been applied to more complex genomes. Usually this involves creating a physical map and a contig (line of overlapping clones) of clones containing a large amount of DNA in a vector such as a YAC, which are then shotgun cloned into smaller vectors and sequenced. However, a whole genome shotgun approach has been used to sequence the mouse, fly and human genomes by the private company Celera. This involves shotgun cloning the whole genome and sequencing the clones without creating a physical map. It is faster and cheaper than creating a physical **gene** map and sequencing clones one by one, but the reliability of reassembling all the sequences of the small fragments into one genomic sequence has been doubted. For example, a part of the fly genome was sequenced by the one-by-one approach and the whole genome shotgun method. The two sequences were compared, and showed differences. 60% of the genes were identical, 31% showed minor differences and 9% showed major differences. The whole genome shotgun method generated the sequence much more quickly, but the one-by-one approach is probably more accurate because the genes were studied in more detail.

See also Cloning, application of cloning to biological problems; Yeast artificial chromosome (YAC); Yeast genetics

SIGNAL HYPOTHESIS

The signal hypothesis was proposed to explain how proteins that were destined for export from **bacteria** or for targeting to certain regions within eucaryotic **microorganisms** (e.g., **yeast**) achieved their target. The hypothesis was proposed in the 1970s by Günter Blobel, who was then as now a molecular biologist at the Rockefeller University in New York. Blobel's work received the 1999 Nobel Prize in medicine or physiology.

The signal hypothesis proposes that proteins destined for secretion, which involves the movement of the protein across a biological membrane, are originally manufactured with an initial sequence of amino acids that may or may not present in the mature protein.

Work by Blobel and others over two decades established the validity of the proposal. The so-called signal sequence is now known to be only some 20 amino acids in length. The arrangement of amino acids in the signal sequence is not random. Rather, the beginning of the sequence, along with a few amino acid residues in the center of the sequence, is comprised of amino acids that are hydrophilic ("water-loving"). Sandwiched between these regions is a central portion that is made up of amino acids that are **hydrophobic** ("water-hating").

The hydrophilic beginning of the signal sequence, which emerges first as the protein is made, associates with the inner hydrophilic surface of the membrane. As the hydrophilic region of the protein merges, it burrows into the core of the membrane bilayer. The short hydrophilic stretch within the signal sequence anchors in the hydrophilic region on the opposite side of the membrane. Thus, the sequence provides an anchor for the continued extrusion of the emerging protein. In some proteins, the signal sequence can be enzymatically clipped off the remainder of the protein. Proteins of Gram-negative bacteria that are exported from the inside of the cell to the periplasmic space between the inner and outer membranes are examples of such processed proteins. Alternatively, the protein may remain anchored to the membrane via the embedded signal sequence.

The signal hypothesis has been demonstrated in plant cells, animal cells, single-celled **eukaryotes** (e.g., yeast), and in bacteria. The malfunction of the signal mechanism can be detrimental in all these systems. In contrast, the use of signal sequences has proven beneficial for the export of bio-engineered drugs from bacteria.

See also Bacterial membranes and cell wall; Prokaryotic membrane transport

SINSHEIMER, ROBERT LOUIS (1920-)

American molecular biologist and biophysicist

Born in Washington, D.C., Robert Sinsheimer attended secondary school in Chicago before studying at the Massachusetts Institute of Technology (MIT). At MIT Sinsheimer took his undergraduate degree in quantitative biology before moving on to complete his Ph.D. in biophysics. Sinsheimer initially accepted a faculty position at MIT but moved to Iowa State College in 1949 to take up the post of professor of biophysics.

Sinsheimer became a professor of biophysics at the California Institute of Technology (Caltech) in 1957 and was Chairman of the Caltech Division of Biology from 1968 to 1977. During this period he conducted a series of investigations into the physical and genetic characteristics of a **bacteriophage** called Phi X 174. These breakthrough studies illuminated the viral genetic processes. Sinsheimer and his colleagues also succeeded for the first time in isolating, purifying, and synthetically replicating viral **DNA**.

The bacteriophage Phi X 174 was an ideal candidate for study because it contained only a single strand of DNA comprised of about 5,500 nucleotides forming approximately 11 genes. In addition it was easier to obtain samples of the bacteriophage DNA.

In 1977 Sinsheimer left Caltech to become a chancellor of the University of California, Santa Cruz. One reason the position of chancellor appealed to him was that it provided a forum to address his concerns that had developed concerning the social implications and potential hazards of recombinant DNA technology and **cloning** methods. Sinsheimer was one of the first scientists to question the potential hazardous uses of **molecular biology** and the ethical implications of the developing technologies. In addition Sinsheimer became committed to promoting scientific literacy among non-scientists.

His early years at Santa Cruz were challenging. During his tenure the university re-established itself as a seat of research and academic excellence. Some of Sinsheimer's accomplishments included the establishment of the Keck telescope, the establishment of programs in agroecology, applied economics, seismological studies, and a major in computer engineering.

Sinsheimer also participated fundamentally in the genesis of the Human Genome Project. In May 1985 Sinsheimer organized a conference at Santa Cruz to consider the benefits of sequencing the human genome. From these and other such deliberations arose the Human Genome Project.

Author of more than 200 scientific papers, Sinsheimer's autobiography, *The Strands of a Life: The Science of DNA and the Art of Education,* was published in 1994.

See also Bacteriophage and bacteriophage typing; Containment and release prevention protocol; Molecular biology and molecular genetics; Phage genetics

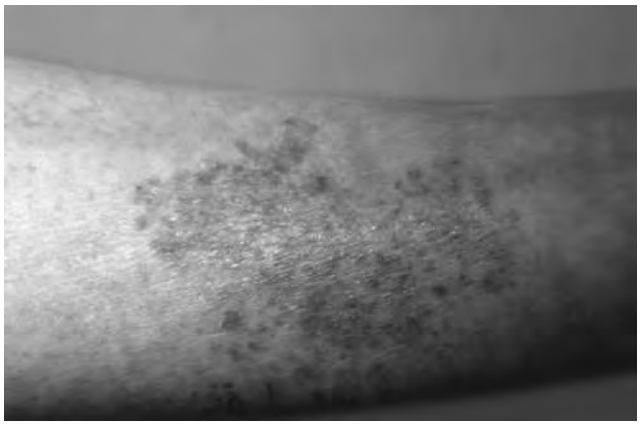
SKIN INFECTIONS

The skin is the largest organ in the human body. It is the front line of defense against many types of pathogens, and remains disease-free over most of its area most of the time. However, breaks in the skin are particularly prone to invasion by **microorganisms**, and skin infections are a relatively common complaint. Skin infections may be bacterial, viral or fungal in nature.

Among the more common bacterial skin infections is impetigo, a usually mild condition caused by staphylococcal or streptococcal **bacteria**. It causes small skin lesions and typically spreads among schoolchildren. Folliculitis results in pustules at the base of hairs or, in more serious cases, in painful boils. Often it is caused by *Staphylococcus* species. A relatively recent manifestation called "hot tub folliculitis" results from *Pseudomonas* bacteria in poorly maintained hot tubs. Those bacterial skin infections that do not resolve spontaneously are treated with topical or oral **antibiotics**.

Among the more serious bacterial infections of the skin is cellulitis, a deep infection involving subcutaneous areas and the lymphatic circulation in the region as well as the skin itself. The affected area is painful, red, and warm to the touch, and the patient may be feverish. Cellulitis is usually caused by bacterial invasion of an injury to the skin. Treatment includes oral and/or intravenous antibiotics, and immobilization and elevation of the affected area.

Viral skin infections typically show up as warts caused by the Human Papillomavirus (HPV). Common warts usually appear on the extremities, especially in children and adolescents. Plantar warts often grow on the heel or sole of the foot, surrounded by overgrown, calloused skin. When they develop on weight-bearing surfaces such as the heel, plantar warts may become painful. HPV also causes genital warts, or condylo-



Skin infection caused by tinea.

mata, which may increase the risk for cervical or penile cancer. Many methods are used in attempts to remove warts, with varying degrees of success. These include cryotherapy, antiviral agents, application of salicylic acid, surgical removal, and laser treatment.

Skin infections caused by **fungi**, including **yeast**, are called dermatomycoses. A common subcategory consists of the dermatophytoses, caused by *Trichophyton* species. These infections include tinea capitis ("cradle cap"), tinea corporis ("ringworm"), tinea cruris ("jock itch"), and tinea pedis ("athlete's foot"). *Candida,* which often affects the mucous membranes, may also be responsible for skin infections. Obese patients are prone to fungal infections in skin folds, as are uncircumcised men. Candida is also involved in some cases of diaper rash. Fungal infections are typically treated with topical imidazole creams or sprays.

See also Bacteria and bacterial infection; Candidiasis; Infection and resistance; Viruses and responses to viral infection; Yeast, infectious

SLEEPING SICKNESS

Sleeping sickness (trypanosomiasis) is a protozoan infection passed to humans through the bite of the tsetse fly. It progresses to death within months or years if left untreated. Near-control of trypanosomiasis was achieved in the 1960s, but the disease has since re-emerged in Sub-Saharan Africa, where political instability and war have hampered **public health** efforts. As of 2002, the **World Health Organization**, in conjunction with Médicines Sans Frontièrs (Doctors Without Borders) and major pharmaceutical companies were in the midst of a five-year major effort to halt the spread of trypanosomiasis and treat its victims.

Protozoa are single-celled organisms considered to be the simplest animal life form. The protozoa responsible for sleeping sickness are a flagellated variety (flagella are hair-like projections from the cell which aid in mobility) which exist only in Africa. The type of protozoa causing sleeping sickness in humans is referred to as the *Trypanosoma brucei* complex. It is divided further into Rhodesian (Central and East Africa) and Gambian (Central and West Africa) subspecies.

The Rhodesian variety live within antelopes in savanna and woodland areas, causing no disruption to the antelope's health. (While the protozoa cause no illness in antelopes, they are lethal to cattle who may become infected.) The protozoa are acquired by tsetse flies who bite and suck the blood of an infected antelope or cow. Within the tsetse fly, the protozoa cycle through several different life forms, ultimately migrating to the salivary glands of the tsetse fly. Once the protozoa are harbored in the salivary glands, they can be deposited into the bloodstream of the fly's next blood meal.

Humans most likely to become infected by Rhodesian trypanosomes are game wardens or visitors to game parks in East Africa. The Rhodesian variety of sleeping sickness causes a much more severe illness with a greater likelihood of eventual death. The Gambian variety of Trypanosoma thrives in tropical rain forests throughout Central and West Africa, does not infect game or cattle, and is primarily a threat to people dwelling in such areas. It rarely infects visitors.

The first sign of sleeping sickness may be a sore appearing at the tsetse fly bite spot about two to three days after having been bitten. Redness, pain, and swelling occur. Two to three weeks later, Stage I disease develops as a result of the protozoa being carried through the blood and lymphatic circulations. This systemic (meaning that symptoms affect the whole body) phase of the illness is characterized by a high fever that falls to normal then re-spikes. A rash with intense itching may be present, and headache and mental confusion may occur. The Gambian form includes extreme swelling of lymph tissue, enlargement of the spleen and liver, and swollen lymph nodes. Winterbottom's sign is classic of Gambian sleeping sickness; it consists of a visibly swollen area of lymph nodes located behind the ear and just above the base of the neck. During this stage, the heart may be affected by a severe inflammatory reaction, particularly when the infection is caused by the Rhodesian form.

Many of the symptoms of sleeping sickness are actually the result of attempts by the patient's **immune system** to get rid of the invading organism. The overly exuberant cells of the immune system damage the patient's organs, causing anemia and leaky blood vessels. These leaky blood vessels help to spread the protozoa throughout the patient's body.

One reason for the immune system's intense reaction to the Trypanosomes is also the reason why the Trypanosomes survive so effectively. The protozoa are able to change rapidly specific markers on their outer coats. These kinds of markers usually stimulate the host's immune system to produce immune cells specifically to target the markers and allow quick destruction of these invading cells. Trypanosomes are able to express new markers at such a high rate of change that the host's immune system cannot catch up.

Stage II sleeping sickness involves the nervous system. The Gambian strain has a clearly delineated phase in which the predominant symptomatology involves the brain. The patient's speech becomes slurred, mental processes slow, and he or she sits and stares or sleeps for long periods of time.

The trypanosome that causes sleeping sickness is commonly transferred to humans by mosquitoes.

Other symptoms resemble Parkinson's disease: imbalance when walking, slow and shuffling gait, trembling of the limbs, involuntary movement, muscle tightness, and increasing mental confusion. These symptoms culminate in coma, then death.

Diagnosis of sleeping sickness can be made by microscopic examination of fluid from the site of the tsetse fly bite or swollen lymph nodes for examination. A method to diagnose Rhodesian trypanosome involves culturing blood, bone marrow, or spinal fluid. These cultures are injected into rats to promote the development of blood-borne protozoan infection. This infection can be detected in blood smears within one to two weeks.

Medications effective against the *Trypanosoma brucei* complex protozoa have significant potential for side effects. Suramin, eflornithine, pentamidine, and several drugs which contain arsenic (a chemical which is potentially poisonous) are effective anti-trypanosomal agents. Each of these drugs requires careful monitoring to ensure that they do not cause serious complications such as a fatal hypersensitivity reaction, kidney or liver damage, or **inflammation** of the brain. Trials are underway to monitor the effectiveness of new medications for treatment of trypanosomiasis.

Prevention of sleeping sickness requires avoiding contact with the tsetse fly; insect **repellents**, mosquito netting, and clothing that covers the limbs to the wrists and ankles are mainstays. There are currently no immunizations available to prevent sleeping sickness.

See also Protists

SLIME LAYER • *see* GLYCOCALYX

SLIME MOLDS

Slime molds are organisms in two taxonomic groups, the cellular slime molds (Phylum Acrasiomycota) and the plasmodial slime molds (Phylum Myxomycota). Organisms in both groups are eukaryotic (meaning that their cells have nuclei) and are fungus-like in appearance during part of their life cycle. For this reason, they were traditionally included in **mycology** textbooks. However, modern biologists consider both groups to be only distantly related to the **fungi**. The two groups of slime molds are considered separately below.

Species in the cellular slime **mold** group are microscopic during most stages of their life cycle, when they exist as haploid (having one copy of each chromosome in the **nucleus**), single-celled amoebas. The amoebas typically feed on **bacteria** by engulfing them, in a process known as **phagocytosis**, and they reproduce by mitosis and fission. Sexual reproduction occurs but is uncommon. Most of what we know about this group is from study of the species *Dictyostelium discoideum*. When there is a shortage of food, the individual haploid amoebas of a cellular slime mold aggregate into a mass of cells called a pseudoplasmodium. A pseudoplasmodium typically contains many thousands of individual cells. In contrast to the plasmodial slime molds, the individual cells in a pseudoplasmodium maintain their own plasma membranes during aggregation. The migrating amoebas often form beautiful aggregation patterns, which change form over time.

After a pseudoplasmodium has formed, the amoebas continue to aggregate until they form a mound on the ground surface. Then, the mound elongates into a "slug." The slug is typically less than 0.04 in (1 mm) in length and migrates in response to heat, light, and other environmental stimuli.

The slug then develops into a sporocarp, a fruiting body with cells specialized for different functions. A sporocarp typically contains about 100,000 cells. The sporocarp of *Dictyostelium* is about 0.08 in (2 mm) tall and has cells in a base, stalk, and ball-like cap. The cells in the cap develop into asexual reproductive spores, which germinate to form new amoebas. The different species of cellular slime molds are distinguished by sporocarp morphology.

Dictyostelium discoideum has been favored by many biologists as a model organism for studies of development, **biochemistry**, and genetics. Aspects of its development are analogous to that of higher organisms, in that a mass of undifferentiated cells develops into a multicellular organism, with different cells specialized for different functions. The development of *Dictyostelium* is much easier to study in the laboratory than is the development of higher organisms.

A food shortage induces aggregation in *Dictyostelium*. In aggregation, individual amoebas near the center of a group of amoebas secrete pulses of cAMP (cyclic adenosine-3'5'-monophosphate). The cAMP binds to special receptors on the plasma membranes of nearby amoebas, causing the cells to move toward the cAMP source for about a minute. Then, these amoebas stop moving and in turn secrete cAMP, to induce other more distant amoebas to move toward the developing aggregation. This process continues until a large, undifferentiated mass of cells, the pseudoplasmodium, is formed.

Interestingly, cAMP is also found in higher organisms, including humans. In *Dictyostelium* and these higher organisms, cAMP activates various biochemical pathways and is synthesized in response to hormones, neurotransmitters, and other stimuli.

The plasmodial slime molds are relatively common in temperate regions and can be found living on decaying plant matter. There are about 400 different species. Depending on the species, the color of the amorphous cell mass, the **plasmodium**, can be red, yellow, brown, orange, green, or other colors. The color of the plasmodium and the morphology of the reproductive body, the sporocarp, are used to identify the different species.

The plasmodial slime molds are superficially similar to the cellular slime molds. Both have a haploid amoeba phase in when cells feed by phagocytosis, followed by a phase with a large amorphous cell mass, and then a reproductive phase with a stalked fruiting body.

However, the plasmodial slime molds are distinguished from the cellular slime molds by several unique features of their life cycle. First, the germinating spores produce flagellated as well as unflagellated cells. Second, two separate haploid cells fuse to produce a zygote with a diploid nucleus. Third, the zygote develops into a plasmodium, which typically contains many thousands of diploid nuclei, all surrounded by a continuous plasma membrane.

The **cytoplasm** of the plasmodium moves about within the cell, a process known as cytoplasmic streaming. This is readily visible with a **microscope**. The function of cytoplasmic streaming is presumably to move nutrients about within the giant cell.

In nature, plasmodial slime molds grow well in wet and humid environments, and under such conditions the plasmodium of some species can be quite large. After a particularly wet spring in Texas in 1973, several residents of a Dallas suburb reported a large, moving, slimy mass, which they termed "the Blob." One reporter in the local press speculated that the Blob was a mutant bacterium, able to take over the earth. Fortunately, a local mycologist soberly identified the Blob as *Fuligo septica,* a species of plasmodial slime mold.

Another plasmodial slime mold, *Physarum polycephalum,* is easily grown in the laboratory and is often used by biologists as a model organism for studies of cytoplasmic streaming, biochemistry, and cytology. The plasmodium of this species moves in response to various stimuli, including ultraviolet and blue light. The proteins actin and myosin are involved in this movement. Interestingly, actin and myosin also control the movement of muscles in higher organisms, including humans.

See also Mycology

SLOW VIRUSES

Historically, the term "slow virus infections" was coined for a poorly defined group of seemingly viral diseases which were later found to be caused by several quite different conventional **viruses**, also unconventional infectious agents. They nevertheless shared the properties of causing diseases with long incubation periods and a protracted course of illness, affecting largely the central nervous and/or the lymph system and usually culminating in death. The slow virus concept was first introduced by the Icelandic physician Bjorn Sigurdsson (1913–1959) in 1954. He and his co-workers had made pioneering studies on slow diseases in sheep including maedi-visna and scrapie. Maedi is a slowly progressive interstitial **pneumonia** of adult sheep while visna is a slow, progressive encephalomyelitis and the same virus, belonging, to the lentivirus subgroup of **retroviruses**, was found to be responsible for both conditions.

Since the original isolation of the maedi-visna virus, concern with slow viral infections, both in animals and in humans, has grown. Research on sheep lentiviruses and their pathogenesis has continued to this day and received an important impetus in the 1980s with the recognition of the devastating condition in humans known as acquired **immunodeficiency** syndrome (**AIDS**). AIDS shared many of the attributes of slow virus infections in animals and led virologists to suspect, then to identify, the lentivirus causing AIDS: the **human immunodeficiency virus** or **HIV**. Questions posed by Bjorn Sigurdsson's work on maedi-visna also became the central pathogenic questions of HIV disease. For example: how and where does HIV persist despite an initially robust and long-sustained immune response? How does HIV actually destroy the tissues it infects? Why do these events unfold so slowly? Final answers to all these questions have still not been found and there is much research still to be done on the lentiviruses but Sigurdsson's contribution to HIV research through the study of maedi-visna is now recognized.

Other slow virus infections of humans due by conventional viruses include progressive multifocal leukoencephalopathy (PML) caused by the JC papovavirus. This is an opportunistic infection in hosts that have defective cell-mediated **immunity** and the majority of human cases now occur in HIV 1 infected individuals. Patients present with progressive multifocal signs including visual loss, aphasia (difficulty speaking), seizures, dementia, personality changes, gait problems, and less commonly, cerebellar, brain stem, and spinal cord features. Death occurs within weeks to months of clinical onset. Subacute sclerosing panencephalitis (SSPE), another slow infection, has been identified as a rare consequence of chronic persistant infection by the **measles** (rubella) virus, causing an insidious syndrome of behavioral changes in young children. Patients develop motor abnormalities, in particular myoclonic jerks, and ultimately become mute, quadriplegic, and in rigid stupor. SSPE is found worldwide with a frequency of one case per million per year. Progressive rubella panencephalitis is another very rare slow virus infection of children and young people caused by the same virus. Most patients have a history of congenital or acquired rubella and the clinical course is more protracted than in SSPE with progressive neurologic deficit occurring over several years. A third slow virus of humans that has had some publicity in recent years is the **human T-cell leukemia virus** (**HTLV**) types 1 and 2 which are associated with adult T-cell leukemia. It was initially thought that the causative agent of AIDS was related to HTLV though it later became clear that whereas HTLV 1 and 2 are both oncogenic ("cancer producing") retroviruses, HIV belongs to the lentivirus sub-group.

An unconventional agent causing slow infections has now been identified as a non-viral "proteinaceous, infectious" agent, or prion. **Prions** give rise to the group of diseases now called transmissible spongiform encephalopathies. In animals these include scrapie in sheep and bovine spongiform encephalopathy (BSE) in cows. Human prion infections include rare dementing diseases like kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker (GSS) syndrome, and fatal familial insomnia (FFI). The prion agent is replicated without provoking any **antibody** response, appears not to have any recognizable nucleic acid component, and is resistant to conventional inactivation techniques for infectious agents. Current evidence suggests that the prion protein is an abnormal isoform of a normal host encoded protein known as PrP, which is coded on the short arm of chromosome 20. Prions appear to "replicate" by a novel form of protein-protein information transfer, the abnormal PrP seemingly inducing the normal protein to undergo a structural change into the abnormal form. Most pathological changes observed with transmissible spongiform encephalopathies are confined to the brain; however, scrapie-induced disorders of the pancreas have also been described. The neuropathology sometimes shows a dramatic spongiform disruption of brain tissue but may also be subtle and non-characteristic, even at the terminal stages of the disease. In the latter cases, diagnosis has to rely on features like clinical signs, transmissibility, detection of abnormal PrP or identification of **mutations** in the PrP **gene**.

In humans, prion diseases may be sporadic, acquired or inherited. Iatrogenic transmissions of the prion have occurred following medical procedures such as pituitary growth hormone injections, where the hormone source was contaminated with prion tissue, or corneal transplants, where a patient accidentally received an infected cornea. The first recognized human prion disease was kuru, which emerged among the South Fore people of New Guinea and is now generally thought to have been transmitted by the practice of ritual cannibalism. CJD is today the most common human prion disease occurring worldwide with a frequency of about one per million per year. The peak incidence occurs in older people between the ages 55 and 65 although recently a "new variant" has emerged in the U.K., which affects individuals at a much earlier age. It is widely believed that the new variant CJD is closely related to the variety causing BSE in cattle and may be contracted by the ingestion of infected beef.

Inherited prion disease can arise from specific point mutations in the PrP gene. Perhaps 10–15% of CJD cases are familial with an autosomal dominant pattern of inheritance. Gerstmann-Straussler-Scheinker syndrome is another rare familial condition that is vertically transmitted in an apparently autosomal dominant way. As with other prion diseases it can be horizontally transmitted to non-human primates and rodents through intracerebral inoculation of brain homogenates from patients with the disease. The exact incidence of the syndrome is unknown but is estimated to be between one and ten per hundred million per year and the condition appears to be an allelic variant of familial Creutzfeldt-Jakob disease. Fatal familial insomnia is the third most common inherited human prion disease. The region of the brain most affected in this condition is the thalamus which monitors sleep patterns. The symptoms of the disease are characterized by progressive insomnia and, as with other prion diseases, eventual motor signs.

See also Viral genetics

SMALLPOX

Smallpox is an infection caused by the **variola virus**, a member of the poxvirus family. Throughout history, smallpox has caused huge **epidemics** resulting in great suffering and enormous death tolls worldwide. In 1980, the **World Health Organization** (**WHO**) announced that a massive program of **vaccination** against the disease had resulted in the complete eradication of the virus (with the exception of stored virus stocks in two laboratories).

Smallpox is an extraordinarily contagious disease. The virus can spread by contact with victims, as well as in contaminated air droplets and even on the surfaces of objects used by other smallpox victims (books, blankets, etc.). After acquisition of the virus, there is a 12–14 day incubation period, during which the virus multiplies, but no symptoms appear. The onset of symptoms occurs suddenly and includes fever and chills, muscle aches, and a flat, reddish-purple rash on the chest, abdomen, and back. These symptoms last about three days, after which the rash fades and the fever drops. A day or two later, the fever returns, along with a bumpy rash starting on the feet, hands, and face. This rash progresses from the feet along the legs, from the hands along the arms, and from the face down the neck, ultimately reaching and including the chest, abdomen, and back. The individual bumps, or papules, fill with clear fluid, and, over the course of 10–12 days, became pus-filled. The pox eventually scabs over, and when the scab falls off it leaves behind a pock-mark or pit, which remains as a permanent scar on the skin of the victim.

Death from smallpox usually follows complications such as **bacterial infection** of the open skin lesions, **pneumonia**, or bone infections. A very severe and quickly fatal form of smallpox was "sledgehammer smallpox," and resulted in hemorrhage from the skin lesions, as well as from the mouth, nose, and other areas of the body. No treatment was ever discovered for smallpox nor could anything shorten the course of the disease. Up until its eradication, smallpox was diagnosed most clearly from the patients' symptoms. **Electron microscopic** studies could identify the variola virus in fluid isolated from disease papules, from infected urine, or from the blood prior to the appearance of the papular rash.

Smallpox is an ancient disease. There is evidence that a major epidemic occurred towards the end of the eighteenth Egyptian dynasty. Studies of the mummy of Pharaoh Ramses V (d. 1157 B.C.) indicate that he may have died of smallpox. Several historical accounts, some dating to the sixth century, describe how different peoples attempted to vaccinate against smallpox. In China, India, and the Americas, from about the tenth century, it was noted that individuals who had even a mild case of smallpox could not be infected again. Material

from people ill with smallpox (e.g., fluid or pus from the papules) was scratched into the skin of those who had never had the illness, in an attempt to produce a mild reaction and its accompanying protective effect. These efforts often resulted in full-fledged smallpox, and sometimes served only to effectively spread the infection throughout the community. In Colonial America, such crude vaccinations against smallpox were outlawed because of the dangers.

In 1798, **Edward Jenner** (1749–1823) published a paper in which he discussed an important observation that milkmaids who contracted a mild infection of the hands (caused by vaccinia virus, a relative of variola) appeared to be immune to smallpox. He created an **immunization** against smallpox that used the pus material found in the lesions of **cowpox** infection. Jenner's paper, although severely criticized at first, later led to much work in the area of vaccinations. Vaccination using Jenner's method proved instrumental in decreasing the number of smallpox deaths.

Smallpox is dangerous only to human beings. Animals and insects can neither be infected by smallpox, nor carry the virus in any form. Humans also cannot carry the virus unless they are symptomatic. These important facts entered into the decision by the WHO to attempt worldwide eradication of the smallpox virus. The methods used in the WHO eradication program were simple and included the careful surveillance of all smallpox infections worldwide to allow for quick diagnosis and immediate quarantine of patients. It also included the immediate vaccination of all contacts of any patient diagnosed with smallpox infection. The WHO program was extremely successful, and the virus was declared eradicated worldwide in May of 1980. Two laboratories (in Atlanta, Georgia and in Koltsovo, Russia) retain samples of the smallpox virus, because some level of concern exists that another poxvirus could mutate (undergo genetic changes) and cause human infection. Other areas of concern include the possibility of smallpox virus being utilized in a situation of **biological warfare**, or the remote chance that the smallpox virus could somehow escape from the laboratories where it is being stored. For these reasons, large quantities of **vaccine** are stored in different countries around the world, so that response to any future threat by the smallpox virus can be prompt.

See also Smallpox, eradication, storage, and potential use as a bacteriological weapon; Vaccine

SMALLPOX: ERADICATION, STORAGE, AND POTENTIAL USE AS A BACTERIOLOGICAL WEAPON

Historically, **smallpox** was one of the most feared diseases in the ancient world. After an extensive and successful eradication program, the **World Health Organization** (**WHO**) certified the global eradication of smallpox infection in 1980. There has not been a single reported case of smallpox infection in over 20 years. However, smallpox was once a deadly disease with the power to decimate populations. Successful efforts to prevent the spread of smallpox through **vaccination** changed the course of Western medicine and indeed, the history of smallpox is a fascinating testament to the effect of health and disease on the development of modern civilization. Today it is difficult to imagine the devastating effects of the disease on the human population. In 1981, smallpox was removed from the WHO list of diseases covered under the International Health Regulations, which detail notification requirements and measures that should be taken to contain an outbreak. The last reported case of smallpox occurred in Somalia in 1977, and on May 8, 1980, the WHO declared the global eradication of smallpox. This meant that smallpox vaccination was no longer required and the WHO indicated that only "investigators at special risk" should have the **vaccine**. It was also decided that seed lots of vaccinia virus would be maintained as well as stocks of 200 million doses of prepared vaccine in case of an accidental outbreak. There is a 30% case-fatality rate associated with smallpox infections among unvaccinated individuals and routine vaccinations have now not been performed in the United States in over 25 years. The fact that stocks of smallpox still exist means that an accidental or deliberate release of the virus could occur. Smallpox, if used as a biological weapon, clearly presents a threat to both civilian and military populations. Thus, although there is little risk of naturally occurring smallpox infections at this time, there is a significant potential for a smallpox epidemic of manmade origin.

The concept of using the variola (smallpox) virus in warfare is an old one. During the French and Indian Wars (1754–1767), British colonial commanders distributed blankets that were used by smallpox victims in order to initiate an epidemic among Native Americans. The mortality rate associated with these outbreaks was as high as 50% in certain tribes. More recently, in the years leading up to World War II, the Japanese military explored smallpox weaponization during operations of Unit 731 in Mongolia and China.

There are a number of characteristics that make the **variola virus** an excellent candidate for use as a biological weapon. An aerosol suspension of variola can spread widely and have a very low infectious dosage. In general, the dissemination of a pathogen by aerosol droplets is the preferred deployment method for biological weapons. Smallpox is highly contagious and is spread through droplet inhalation or ingestion. As there are no civilian or military smallpox vaccination requirements at this time, a large susceptible population is at risk from the infection. The incubation period in naturally occurring cases averages seven to 14 days. However, the period could be shortened to three to seven days, especially in the cases of aerosol application. People who have contracted the disease are contagious during the late stages of the incubation period, even though they remain asymptomatic. Thus, transmission of the disease can occur as early as two days after exposure to the virus. Depending on the climate, corpses of smallpox victims remain infectious for days to months. The duration of the disease is long and coupled with the complex isolation and protection requirements of smallpox treatment, each infected person would require the efforts of several medical support personnel.

In general, the worldwide practice of smallpox **immunization** greatly diminished the fear of an epidemic caused by a deliberate release of the virus. Although the disease was declared eradicated in 1980, stores of smallpox officially exist at the two WHO-approved repositories. The first is at the **Centers for Disease Control** and Prevention in Atlanta, USA. The second is the State Research Center for **Virology** and **Biotechnology** (also known as Vector) at Koltsovo, in the Novosibirsk region of Siberian Russia. In June 1995, WHO inspected the Koltsovo facility and determined that it was an acceptable storage facility after the virus stocks were moved there from the original storage site at the Institute of Viral Preparations in Moscow. All other laboratories around the world were required to destroy their remaining stores of smallpox virus. Concomitantly, WHO recommended that all countries discontinue vaccination against the disease.

Despite the provisions of the WHO and the 1972 **Biological Weapons Convention**, the former Soviet Union maintained a sophisticated and large-scale research and development program for biological weapons implementation. This research was carried out at both military and civilian level. It is now known that the Soviet Union successfully developed and adapted smallpox virus for use in strategic weapons.

Considerable debate has ensued regarding the officially remaining stores of smallpox virus. The WHO Ad hoc Committee on Orthopoxvirus Infections has, since 1986, consistently recommended destruction of the remaining reserves of the smallpox virus. The initial proposal was to destroy the remaining stocks in December 1990. However, the possibility that smallpox has been, and might be, incorporated into biological weapons has encouraged the scientific community to continue research on the pathogen. Although the tentative date set for the destruction of all remaining smallpox stores is late 2002, a consensus among scientists and military strategists has not yet been reached and in view of current political unrest in areas such as the Middle East, total destruction of all stores is not likely to happen.

Since many laboratories involved in biological weapons research and development in the former Soviet Union are now working with decreased funding, staff, and support, there is concern that bioweapons resources and expertise may spread to other countries. A report from the Washington Center for Strategic and International Studies states that at least 10 countries are involved in biological weapon research programs. The ability of a group to acquire variola and develop it as a biological weapon is limited by several factors. Specialized skills are required to grow smallpox in effectively large quantities and to adapt it for use as an aerosol-based weapon. It is unlikely that small, technically limited facilities or dissident groups would use smallpox as a weapon. Also, the open use of a biological weapon by any nation or political state would undoubtedly elicit severe retaliation. Lastly, the smallpox virus is not as readily available as other agents of biological terrorism, such as **anthrax** (*Bacillus anthraci*) or plague (*Yersinia pestis).* Therefore, analysis of these and other factors have led bioweapons experts to conclude that well-financed and highly organized private groups or politically/state financed terrorist groups would be the most likely to use smallpox as a bio-weapon.

See also Bioterrorism; Bioterrorism, protective measures; Epidemics, viral; Viruses and responses to viral infection

SNOW BLOOMS

Snow bloom refers to the rapid growth and increase in numbers of so-called snow algae on the surface and interior of snow fields. Typically occurring as the surface of the snow warms in the springtime sun, the algal growth confers various colors to the snow. Colors of different algal species include yellow, red, green, and orange.

Blooms occur when nutrients are abundant and conditions such as temperature are conducive to rapid growth. "Red tide," due to the growth of a **diatoms** in salt water, is another example of a bloom.

There are some 350 species of snow algae. A common species is *Chlamydomonas nivalis*.

Snow algae have been known for millennia. The Greek philosopher Aristotle described red snow over 2,000 years ago. The algal basis for the blooms was determined in the early nineteenth century, when some red-colored snow obtained by a British expedition near Greenland was analyzed.

Snow blooms occur most frequently in high altitude areas where snow persists over a long time and accumulates to great depths. Examples include the Sierra Nevada range in California and the Rocky Mountains of North America.

The various colors of snow blooms reflect the presence of various pigmented compounds in the algae. These compounds, which are called carotenoids, confer protection against the sunlight, particularly against the ultraviolet portion of the spectrum. Red algae are more sunlight tolerant than are green, orange, and yellow-pigmented algae. Non-red algae tend to shield themselves from the sunlight by growing beneath the snow's surface.

In another adaptation, the algal membrane is adapted for cold, in much the same way that cold-loving **bacteria** are, via the presence of lipids that remain pliable at low temperatures.

Another feature of snow algae that contributes to their tolerance is their ability to form cysts. These are analogous to bacterial spores, in that they provide the algae with a means of becoming metabolically dormant during inhospitable periods. During the winter, the cysts remain encased in snow. Indeed, experiments have determined that cysts are not inactivated even after a prolonged storage at -94° F (-70° C). As the snow melts in the springtime, resuscitation of the cysts occurs. The algal cells migrate to the surface of the snow in order to reproduce. After reproducing the cells drift down into the nutritionally poor subsurface, where their **transformation** into cyst form is again stimulated. The following spring the cycle is repeated.

See also Psychrophilic bacteria

SOIL FORMATION, INVOLVEMENT OF MICROORGANISMS

Microorganisms are essential to soil formation and soil ecology because they control the flux of nutrients to plants (i.e., control of carbon, nitrogen, and sulfur cycles,), promote nitrogen fixation, and promote soil detoxification of inorganic and naturally occurring organic pollutants. Soil microorganisms are also part of several food chains, thus serving as source nutrients to one another, and frequently serve as the primary members of food chains in soil biota.

The roots of plants are also part of soil biota and some **fungi**. Many **bacteria** live in symbiotic relation to plant roots, around which there is an area of elevated microbial activity, known as rhizosphere. The Animalia kingdom is also represented in soil biota by Nematodes, Earthworms, Mollusks, Acarina, Collembola, as well as several insects and larvae that feed mostly on decaying organic matter. They all take part in the soil food chain and help to promote the conversion of organic matter into bacterial and fungal biomass. Soil microbiology is a relatively recent discipline and it is estimated that about only one percent of soil microorganisms are so far identified.

The soil ecosystem is composed of inorganic matter (calcium, iron oxide, nitrates, sulfur, phosphates, ash, and stone particles), substrates (fallen leaves, dead organisms, rotten wood, dead roots), organisms (microbes, animals, and plants), air, and water. Bacteria and fungi are mostly heterotrophic organisms that feed on the existing organic matter by decomposing them in order to absorb the resultant micronutrients and minerals. Therefore, they are essential to the recycling process of nutrients that keeps soils in good condition for plant growth. The community of microorganisms in a given type of soil differs from that belonging to another soil type. They are highly dependent on environmental factors such as levels of carbon dioxide, oxygen, hydrogen, soil **pH** (whether acid, alkaline, or neutral), types of substrates, amounts of available substrates, levels of moisture, and temperature. Each community is highly complex, and so far, little is known about the succession of microorganisms in the food chains and the interconnected food webs they form, or about the sequence of events in the cycling pathways of soil ecosystem.

The arrival of new substrate in the soil increases bacterial populations that feed on them, thus recycling in the process, nutrients important to both plants and other soil organisms. Bacterial expansion leads to a second event, known as succession, which is the growth of **protozoa** populations that predate bacteria. The expansion of protozoa populations triggers the activity of mites, which feed on protozoans. Substrate arrival triggers as well the activity and expansion of fungi populations, which are also decomposers. Some fungal species compete with other fungal species for the same substrates, such as the *Pisolithus* and the *Fusarium*. Nematodes are triggered into action and feed on both fungi and other species of nematodes. Some fungi are able to entrap and feed on nematodes too. In the rhizospheres, these populations are more active than in other parts of the soil and atmospheric factors may influence rhizospheres biota. An American research group is studying the response of soil biota in California grasslands to determine the long-term effect of increased levels of carbon dioxide on soil biota dynamics and on plant growth. They found that in a carbon dioxide enriched atmospheric environment, the colonization of plant roots by fungi is augmented, which facilitates carbon and nutrient exchange between the host plants and the fungi (i.e., symbiosis), thus favoring fungi colonies to expand within the soil, as well favoring the growth of grass. Consequently, the number of soil micro arthropods has also increased, since many of them feed on fungi colonies. However, after six years of experimental carbon dioxide atmospheric enrichment, significant increases on bacterial populations were not recorded. Therefore, the experiment succeeded in illustrating one portion of the food chain in grassland soil, and supplied evidence that the induced enhancement of natural-occurring symbiotic relationships in the rhizosphere may be useful for agricultural productive purposes.

See also Bacterial kingdoms; Composting, microbiological aspects; Microbial symbiosis; Microbial taxonomy; Photosynthetic microorganisms; Protozoa; Slime molds

SPACE MICROBIOLOGY

• *see* EXTRATERRESTRIAL MICROBIOLOGY

SPECTROPHOTOMETER

A spectrophotometer is an optical device that can determine the concentration of a compound or particles in a solution or suspension.

Light of a pre-selected wavelength is shone through a chamber that houses the sample. The sample particles, **bacteria** for example, will absorb some of the light. The amount of light that is absorbed increases with increasing numbers of bacteria in a predictable way. The relationship between absorbance and the number of absorbing sample molecules is expressed mathematically as the Beer-Lambert Law.

The absorbance of light can also be described as the optical density of the sample solution or suspension.

The percent of light that has been absorbed can be determined and, by comparing this absorption to a graph of the absorption of known numbers of bacteria, the concentration of bacteria in the suspension can be computed. In a microbiology laboratory, such measurements are routinely used in **bacterial growth** studies, to determine the number of bacteria growing in a **culture** at certain times based on the absorbance of the suspension. A standard curve can be constructed that relates the various measured optical densities to the resulting number of living bacteria, as determined by the number of bacteria from a defined portion of the suspensions that grows on **agar** medium.

Some spectrophotometers are equipped with a single measuring chamber. For these so-called single-beam instruments, the absorbance of a sample is taken, followed by the

absorbance of a control. Typically, a bacterial control is uninoculated growth medium, so the absorbance should be zero. In typical growth curve studies, the bacterial culture can be grown in a special flask called a side-arm flask. The side arm is a test tube that can be inserted directly into a spectrophotometer.

Double-beam spectrophotometers are also available and are the norm now in research microbiology laboratories. In these instruments the light beam is split into two beams by means of mirrors. One light path goes through the sample chamber and the other light beam passes through what is referred to as the reference cell or chamber. The ration of the absorbance between the two chambers is computed and is used to determine sample concentration.

Depending on the spectrophotometer, absorbance can be taken at a single wavelength, or scanned through a spectrum of wavelengths. The latter can be a useful means of identifying components of the sample, based on their preferential absorption of certain wavelengths of light.

See also Laboratory techniques in microbiology

SPECTROSCOPY

Because organisms present unique spectroscopic patterns, spectroscopic examination (e.g., Raman spectroscopy) of **microorganisms** (e.g., microbial cells) can help to differentiate between species and strains of microbes. Spectroscopic examination can also aid in the identification and measurement of subcellular processes (e.g., CO_2 production) that facilitate the understanding of cell growth, response to environmental stimuli, and drug actions.

The measurement of the absorption, emission, or scattering of electromagnetic radiation by atoms or molecules is referred to as spectroscopy. A transition from a lower energy level to a higher level with transfer of electromagnetic energy to the atom or molecule is called absorption; a transition from a higher energy level to a lower level results in the emission of a photon if energy is transferred to the electromagnetic field; and the redirection of light as a result of its interaction with matter is called scattering.

When atoms or molecules absorb electromagnetic energy, the incoming energy transfers the quantized atomic or molecular system to a higher energy level. Electrons are promoted to higher orbitals by ultraviolet or visible light; vibrations are excited by infrared light, and rotations are excited by microwaves. Atomic-absorption spectroscopy measures the concentration of an element in a sample, whereas atomic-emission spectroscopy aims at measuring the concentration of elements in samples. UV-VIS absorption spectroscopy is used to obtain qualitative information from the electronic absorption spectrum, or to measure the concentration of an analyte molecule in solution. Molecular fluorescence spectroscopy is a technique for obtaining qualitative information from the electronic fluorescence spectrum, or, again, for measuring the concentration of an analyte in solution.

Infrared spectroscopy has been widely used in the study of surfaces. The most frequently used portion of the infrared spectrum is the region where molecular vibrational frequencies occur. This technique was first applied around the turn of the twentieth century in an attempt to distinguish water of crystallization from water of constitution in solids.

Ultraviolet spectroscopy takes advantage of the selective absorbance of ultraviolet radiation by various substances. The technique is especially useful in investigating biologically active substances such as compounds in body fluids, and drugs and narcotics either in the living body (in vivo) or outside it (in vitro). Ultraviolet instruments have also been used to monitor air and **water pollution**, to analyze dyestuffs, to study carcinogens, to identify food additives, to analyze petroleum fractions, and to analyze pesticide residues. Ultraviolet photoelectron spectroscopy, a technique that is analogous to x-ray photoelectron spectroscopy, has been used to study valence electrons in gases.

Microwave spectroscopy, or molecular rotational resonance spectroscopy, addresses the microwave region and the absorption of energy by molecules as they undergo transitions between rotational energy levels. From these spectra, it is possible to obtain information about molecular structure, including bond distances and bond angles. One example of the application of this technique is in the distinction of trans and gauche rotational isomers. It is also possible to determine dipole moments and molecular collision rates from these spectra.

In nuclear magnetic resonance (NMR), resonant energy is transferred between a radio-frequency alternating magnetic field and a **nucleus** placed in a field sufficiently strong to decouple the nuclear spin from the influence of atomic electrons. Transitions induced between substrates correspond to different quantized orientations of the nuclear spin relative to the direction of the magnetic field. Nuclear magnetic resonance spectroscopy has two subfields: broadline NMR and high resolution NMR. High resolution NMR has been used in inorganic and organic chemistry to measure subtle electronic effects, to determine structure, to study chemical reactions, and to follow the motion of molecules or groups of atoms within molecules.

Electron paramagnetic resonance is a spectroscopic technique similar to nuclear magnetic resonance except that microwave radiation is employed instead of radio frequencies. Electron paramagnetic resonance has been used extensively to study paramagnetic species present on various solid surfaces. These species may be metal ions, surface defects, or adsorbed molecules or ions with one or more unpaired electrons. This technique also provides a basis for determining the bonding characteristics and orientation of a surface complex. Because the technique can be used with low concentrations of active sites, it has proven valuable in studies of oxidation states.

Atoms or molecules that have been excited to high energy levels can decay to lower levels by emitting radiation. For atoms excited by light energy, the emission is referred to as atomic fluorescence; for atoms excited by higher energies, the emission is called atomic or optical emission. In the case of molecules, the emission is called fluorescence if the transition

occurs between states of the same spin, and phosphorescence if the transition takes place between states of different spin.

In x-ray fluorescence, the term refers to the characteristic x rays emitted as a result of absorption of x rays of higher frequency. In electron fluorescence, the emission of electromagnetic radiation occurs as a consequence of the absorption of energy from radiation (either electromagnetic or particulate), provided the emission continues only as long as the stimulus producing it is maintained.

The effects governing x-ray photoelectron spectroscopy were first explained by Albert Einstein in 1905, who showed that the energy of an electron ejected in photoemission was equal to the difference between the photon and the binding energy of the electron in the target. In the 1950s, researchers began measuring binding energies of core electrons by x-ray photoemission. The discovery that these binding energies could vary as much as 6 eV, depending on the chemical state of the atom, led to rapid development of x-ray photoelectron spectroscopy, also known as Electron Spectroscopy for Chemical Analysis (ESCA). This technique has provided valuable information about chemical effects at surfaces. Unlike other spectroscopies in which the absorption, emission, or scattering of radiation is interpreted as a function of energy, photoelectron spectroscopy measures the kinetic energy of the electrons(s) ejected by x-ray radiation.

Mössbauer spectroscopy was invented in the late 1950s by Rudolf Mössbauer, who discovered that when solids emit and absorb gamma rays, the nuclear energy levels can be separated to one part in 10^{14}, which is sufficient to reflect the weak interaction of the nucleus with surrounding electrons. The Mössbauer effect probes the binding, charge distribution and symmetry, and magnetic ordering around an atom in a solid matrix. An example of the Mössbauer effect involves the Fe-57 nuclei (the absorber) in a sample to be studied. From the ground state, the Fe-57 nuclei can be promoted to their first excited state by absorbing a 14.4-keV gamma-ray photon produced by a radioactive parent, in this case Co-57. The excited Fe-57 nucleus then decays to the ground state via electron or gamma ray emission. Classically, one would expect the Fe-57 nuclei to undergo recoil when emitting or absorbing a gamma-ray photon (somewhat like what a person leaping from a boat to a dock observes when his boat recoils into the lake); but according to quantum mechanics, there is also a reasonable possibility that there will be no recoil (as if the boat were embedded in ice when the leap occurred).

When electromagnetic radiation passes through matter, most of the radiation continues along its original path, but a tiny amount is scattered in other directions. Light that is scattered without a change in energy is called Rayleigh scattering; light that is scattered in transparent solids with a transfer of energy to the solid is called Brillouin scattering. Light scattering accompanied by vibrations in molecules or in the optical region in solids is called Raman scattering.

In vibrational spectroscopy, also known as Raman spectroscopy, the light scattered from a gas, liquid, or solid is accompanied by a shift in wavelength from that of the incident radiation. The effect was discovered by the Indian physicist C. V. Raman in 1928. The Raman effect arises from the inelastic scattering of radiation in the visible region by molecules. Raman spectroscopy is similar to infrared spectroscopy in its ability to provide detailed information about molecular structures. Before the 1940s, Raman spectroscopy was the method of choice in molecular structure determinations, but since that time infrared measurements have largely supplemented it. Infrared absorption requires that a vibration change the dipole moment of a molecule, but Raman spectroscopy is associated with the change in polarizability that accompanies a vibration. As a consequence, Raman spectroscopy provides information about molecular vibrations that is particularly well suited to the structural analysis of covalently bonded molecules, and to a lesser extent, of ionic crystals. Raman spectroscopy is also particularly useful in studying the structure of polyatomic molecules. By comparing spectra of a large number of compounds, chemists have been able to identify characteristic frequencies of molecular groups, e.g., methyl, carbonyl, and hydroxyl groups.

See also Biotechnology; Electron microscope, transmission and scanning; Electron microscopic examination of microorganisms; Electrophoresis; Enzyme-linked immunosorbant assay (ELISA); Epidemiology, tracking diseases with technology; Fluorescence in situ hybridization (FISH); Laboratory techniques in immunology; Laboratory techniques in microbiology; Microscope and microscopy

SPHEROPLASTS • *see* PROTOPLASTS AND SPHEROPLASTS

SPINAE • *see* BACTERIAL APPENDAGES

SPIROCHETES

Spirochetes are a group comprised of six genera of **bacteria** in a family known as *Spirochaete*. They are named because of their spiral shape. Typically, spirochetes are very slender. Their length can vary from about five microns (millionths of an inch) to several hundred microns, depending on the species. Under the light or electron **microscope**, the tight coiling that is characteristic of spirochetes is readily visible. Spirochetes are a significant health threat to humans. Both **syphilis** and **Lyme disease** are caused by spirochetes. Beneficially, spirochetes contribute to digestion in ruminants such as cows.

Besides their shape, another distinctive aspect of spirochetes in the presence of what is essentially internal flagella. These structures, called axial filaments, are embedded in the cell wall of the bacterium. They are constructed very similarly as flagella, having the characteristic arrangement of structures that anchors the filament to the cell membrane. There can be only a few to as many as 200 axial filaments present in a given bacterium. The rigidity of an axial filament allows a bacterium to move in a corkscrew type of motion. Axial filaments are present in all spirochetes except *Treponema*.

Spirochetes have varied habitats and growth requirements. Some of the bacteria require oxygen for their survival, while others do not tolerate the presence of oxygen.

In terms of human health, spirochetes are noteworthy because of the disease causing members of the group. *Treponema pallidum* is the cause of syphilis and *Borrelia burgdorferi* is the cause of Lyme disease, which can produce a chronic infection that can result in arthritis, damage to the central nervous system, and even heart failure. *Borrelia burgdorferi* can convert to a metabolically dormant cyst in natural environments and even in humans. The cyst form allows the bacterium to survive inhospitable conditions and to elude host immune defense mechanisms.

In ruminants, spirochetes are beneficial. Their chemical activities help the cow or other ruminant digest food. Spirochetes also live in harmony with mussels and oysters, where the bacteria help in feeding by acting as cilia to sweep food into the mollusk.

A spirochete known as *Aquaspririllum magnetotacticum* is of interest to microbiologists because it is one of a number of bacteria that possess magnetic particles. These particles allow a bacterium to orient itself in the water in relation to Earth's magnetic field.

See also Bacteria and bacterial infection; Bacterial movement; Magnetotactic bacteria

SPONTANEOUS GENERATION THEORY • *see* HISTORY OF MICROBIOLOGY

SPOROZOA

The fifth Phylum of the Protist Kingdom, known as Apicomplexa, gathers several species of obligate intracellular protozoan **parasites** classified as Sporozoa or Sporozoans, because they form reproductive cells known as spores. Many sporozoans are parasitic and pathogenic species, such as *Plasmodium (P. falciparum, P. malariae, P. vivax), Toxoplasma gondii, Pneumocysts carinii, Coccidian, Babesia, Cryptosporidum (C. parvum, C. muris),* and *Gregarian.* The Sporozoa reproduction cycle has both asexual and sexual phases. The asexual phase is termed schizogony (from the Greek, meaning generation through division), in which merozoites (daughter cells) are produced through multiple nuclear fissions. The sexual phase is known as sporogony (i.e., generation of spores) and is followed by gametogony or the production of sexually reproductive cells termed gamonts. Each pair of gamonts form a gamontocyst where the division of both gamonts, preceded by repeated nuclear divisions, originates numerous gametes. Gametes fuse in pairs, forming zygotes that undergo meiosis (cell division), thus forming new sporozoites. When sporozoites invade new host cells, the life cycle starts again. This general description of Sporozoan life cycle has some variation among different species and groups.

Sporozoans have no flagellated extensions for locomotion, with most species presenting only gliding motility, except for male gametes in the sexual phase, which have a flagellated stage of motility. All Sporozoa have a cellular structure known as apical complex, which gave origin to the name of the Phylum, i.e., Apicomplexa. Sporozoa cellular organization consists of the apical complex, micropore, longitudinal microtubular cytoskeleton, and cortical alveoli. The apical complex consists of cytoskeletal and secretory structures forming a conoid (a small open cone), polar wings that fix the cytoskeletal microtubules, two apical rings, and secretory vesicles known as micronemes and rhoptries. The apical complex enables Sporozoans to invade the host cells.

Plasmodium species are the causing agents of **malaria** in humans and animals and affects approximately 300 million people around the world, with an estimative of one million new cases each year. They are transmitted by the female anopheles mosquito (infecting vector) that injects *Plasmodium* sporozoites present in the salivary glands of the mosquito into the host's blood stream. Once in the blood stream, *Plasmodium* sporozoites invade erythrocytes (red blood cells) and migrate to the liver to infect the hepatocytes, where their asexual reproductive phase starts. When the merozoite stage is reached, they are released into the circulation again, where they become ring-like trophozoites that undergo schizogony, forming new merozoites that invade the erythrocytes, thus repeating the reproductive cycle. Female anopheles mosquitoes ingest merozoites together with the host's blood. Ingested merozoites form zygotes in the guts of the vector mosquito, later developing into oocysts, from which new sporozoites will be formed and migrate to the anopheles' salivary gland, ready to contaminate the next host. Malaria can also be transmitted through infected blood transfusions.

The vectors for *Babesia* are ticks, causing fever, peripheral capillary hemorrhage, and anemia. Contaminated cats are *Toxoplasma gondii* direct vectors to humans, through the ingestion of oocysts present in cat feces. However, this parasite is also present in birds and other mammals, and humans can be infected by ingesting raw or poorly cooked contaminated meat. Pregnant women may have miscarriages when infected, or can transmit **toxoplasmosis** through the placenta to the fetus, leading to blindness and/or mental retardation of the child. Periodic fecal tests of the house cat and adequate treatment may prevent transmission, as well as avoidance of half-cooked meat in the diet.

Pneumocysts carinii causes interstitial plasma cell **pneumonia** when the cysts containing trophozoites are inhaled. *Cryptosporidium parvum* is usually transmitted through the ingestion of water or foods contaminated with its oocysts, causing intestinal infection and, in immunodepressed patients, diarrhea can be chronic, accompanied by fever. *Coccidian* species infect epithelial tissues of both vertebrates and invertebrates whereas *Gregarian* species are found in the body cavities of invertebrates, such as earthworms.

See also Gastroenteritis; Malaria and the physiology of parasitic infections; Microbial taxonomy

SPORULATION

Sporulation is the formation of nearly dormant forms of **bacteria**. In a limited number of bacteria, spores can preserve the genetic material of the bacteria when conditions are inhospitable and lethal for the normal (vegetative) form of the bacteria. The commitment of a bacterium to the sporulation process sets in motion a series of events that transform the cell.

Sporulation ultimately provides for a multilayered structure can be maintained for a very long time. Relative to the norm life span of the microorganism, spores are designed to protect a bacterium from heat, dryness, and excess radiation for a long time. Endospores of *Bacillus subtilis* have been recovered from objects that are thousands of years old. Furthermore, these are capable of resuscitation into an actively growing and dividing cell. Spores have been recovered from amber that is more than 250 million years old.

Given that resuscitation is possible, sporulation does not result in a completely inert structure. The interior of a spore contains genetic material, **cytoplasm**, and the necessary **enzymes** and other materials to sustain activity. But, this activity occurs at an extremely slow rate; some 10 million times slower than the metabolic rate of a growing bacterium.

The sporulation process has been well studied in *Bacillus subtilis*. The process is stimulated by starvation. Typically, sporulation is a "last resort," when other options fail (e.g., movement to seek new food, production of enzymes to degrade surrounding material, production of antimicrobial agents to wipe out other microbes competing for the food source, etc.). The genetic grounding for the commitment to form a spore is a protein called SpoA. This protein functions to promote the **transcription** of genes that are required for the conversion of the actively growing bacterium to a spore. The formation of an active SpoA protein is controlled by a series of reactions that are themselves responsive to the environmental conditions. Thus, the activation of SpoA comes only after a number of checkpoints have been passed. In this way a bacterium has a number of opportunities to opt out of the sporulation process. Once committed to sporulation, the process is irreversible.

A similar series of reactions has been identified as a means of regulating the degree of host damage caused by a *Bordetella pertussis*, the bacterium that causes **pertussis**, as well as in the response of the **yeast** *Saccharomyces cervesiae* to osmotic pressure.

Sporulation begins with the duplication of the bacterial genome. The second copy and some of the cytoplasm is then enveloped in an in-growth of the membrane that surrounds the bacterium. The result is essentially a little spherical cell inside the larger bacterium. The little cell is referred to as the "daughter cell" and the original bacterium is now called the "mother cell." Another membrane layer is laid down around the daughter cell. Between these two membranes lies a layer of **peptidoglycan** material, the same rigid material that forms the stress-bearing network in the bacterial cell wall. Finally, a coat of proteins is layered around the outside of the daughter cell. The result is a nearly impregnable sphere.

The above spore is technically termed an endospore, because the formation of the membrane-enclosed daughter cell occurs inside the mother cell. In a so-called exospore, the duplicated **DNA** migrates next to a region on the inner surface of the cell membrane and then a bud forms. As the bud protrudes further outward, the DNA is drawn inside the bud. Examples of endospore forming bacteria include those in the genera *Bacillus* and *Clostridium*. Endospore forming bacteria include *Methylosinus*, *Cyanobacteria*, and *Microsporidia*.

When still in the mother cell, the location of the spore (e.g., in the center, near one end or at one pole) is often a distinctive feature for a particular species of bacteria, and can be used as a feature to identify the bacteria.

As the mother cell dies and degrades, the spore will be freed. When conditions become more hospital, the metabolic machinery within the spore will sense the change and a reverse process will be initiated to transform the spore into a vegetative cell.

The type of sporulation described here is different from the sporulation process that occurs in many kinds of **fungi** and in the bacteria called *Actinomyces*. The latter spores are essentially seeds, and are used in the normal reproduction cycle of the **microorganisms**. Bacterial sporulation is an emergency protective and survival strategy.

See also Asexual generation and reproduction; Bacterial adaptation; Bacterial growth and division; Bacterial kingdoms; Bacterial membranes and cell wall; Cell cycle (prokaryotic), genetic regulation of; Desiccation; Extraterrestrial microbiology; Extremophiles; Fossilization of bacteria; Genetic identification of microorganisms; Genetic regulation of prokaryotic cells; Life, origin of; Radiation resistant bacteria

STANLEY, WENDELL MEREDITH (1904-1971)

American biochemist

Wendell Meredith Stanley was a biochemist who was the first to isolate, purify, and characterize the crystalline form of a virus. During World War II, he led a team of scientists in developing a **vaccine** for viral **influenza**. His efforts have paved the way for understanding the molecular basis of heredity and formed the foundation for the new scientific field of **molecular biology**. For his work in crystallizing the **tobacco mosaic virus**, Stanley shared the 1946 Nobel Prize in chemistry with John Howard Northrop and James B. Sumner.

Stanley was born in the small community of Ridgeville, Indiana. His parents, James and Claire Plessinger Stanley, were publishers of a local newspaper. As a boy, Stanley helped the business by collecting news, setting type, and delivering papers. After graduating from high school he enrolled in Earlham College, a liberal arts school in Richmond, Indiana, where he majored in chemistry and mathematics. He played football as an undergraduate, and in his senior year, he became team captain and was chosen to play end on the Indiana All-State team. In June of 1926, Stanley graduated with a Bachelor

of Science degree. His ambition was to become a football coach, but the course of his life was changed forever when an Earlham chemistry professor invited him on a trip to Illinois State University. Here, he was introduced to Roger Adams, an organic chemist, who inspired him to seek a career in chemical research. Stanley applied and was accepted as a graduate assistant in the fall of 1926.

In graduate school, Stanley worked under Adams, and his first project involved finding the stereochemical characteristics of biphenyl, a molecule containing carbon and hydrogen atoms. His second assignment was more practical; Adams was interested in finding chemicals to treat **leprosy**, and Stanley set out to prepare and purify compounds that would destroy the disease-causing pathogen. Stanley received his master's degree in 1927 and two years later was awarded his Ph.D. In the summer of 1930, he was awarded a National Research Council Fellowship to do postdoctoral studies with Heinrich Wieland at the University of Munich in Germany. Under Wieland's tutelage, Stanley extended his knowledge of experimental **biochemistry** by characterizing the properties of some **yeast** compounds.

Stanley returned to the United States in 1931 to accept the post of research assistant at the Rockefeller Institute in New York City. Stanley was assigned to work with W. J. V. Osterhout, who was studying how living cells absorb potassium ions from seawater. Stanley was asked to find a suitable chemical model that would simulate how a marine plant called *Valonia* functions. Stanley discovered a way of using a water-insoluble solution sandwiched between two layers of water to model the way the plant exchanged ions with its environment. The work on *Valonia* served to extend Stanley's knowledge of biophysical systems, and it introduced him to current problems in biological chemistry.

In 1932, Stanley moved to the Rockefeller Institute's Division of Plant Pathology in Princeton, New Jersey. He was primarily interested in studying **viruses**. Viruses were known to cause diseases in plants and animals, but little was known about how they functioned. Stanley's assignment was to characterize viruses and determine their composition and structure.

Stanley began work on a virus that had long been associated with the field of **virology**. In 1892, D. Ivanovsky, a Russian scientist, had studied tobacco mosaic disease, in which infected tobacco plants develop a characteristic mosaic pattern of dark and light spots. He found that the tobacco plant juice retained its ability to cause infection even after it was passed through a filter. Six years later M. Beijerinck, a Dutch scientist, realized the significance of Ivanovsky's discovery: the filtration technique used by Ivanovsky would have filtered out all known **bacteria**, and the fact that the filtered juice remained infectious must have meant that something smaller than a bacterium and invisible to the ordinary light **microscope** was responsible for the disease. Beijerinck concluded that tobacco mosaic disease was caused by a previously undiscovered type of infective agent, a virus.

Stanley was aware of recent techniques used to precipitate the tobacco mosaic virus (**TMV**) with common chemicals. These results led him to believe that the virus might be a protein susceptible to the reagents used in protein chemistry. He set out to isolate, purify, and concentrate the tobacco mosaic virus. He planted Turkish tobacco plants, and when the plants were about 6 in (15 cm) tall, he rubbed the leaves with a swab of linen dipped in TMV solution. After a few days, the heavily infected plants were chopped and frozen. Later, he ground and mashed the frozen plants to obtain a thick, dark liquid. He then subjected the TMV liquid to various **enzymes** and found that some would inactivate the virus and concluded that TMV must be a protein or something similar. After exposing the liquid to more than 100 different chemicals, Stanley determined that the virus was inactivated by the same chemicals that typically inactivated proteins, and this suggested to him, as well as others, that TMV was protein-like in nature.

Stanley then turned his attention to obtaining a pure sample of the virus. He decanted, filtered, precipitated, and evaporated the tobacco juice many times. With each chemical operation, the juice became more clear and the solution more infectious. The result of two-and-one-half years of work was a clear concentrated solution of TMV that began to form into crystals when stirred. Stanley filtered and collected the tiny, white crystals and discovered that they retained their ability to produce the characteristic lesions of tobacco mosaic disease.

After successfully crystallizing TMV, Stanley's work turned toward characterizing its properties. In 1936, two English scientists at Cambridge University confirmed Stanley's work by isolating TMV crystals. They discovered that the virus consisted of 94% protein and 6% nucleic acid, and they concluded that TMV was a nucleoprotein. Stanley was skeptical at first. Later studies, however, showed that the virus became inactivated upon removal of the nucleic acid, and this work convinced him that TMV was indeed a nucleoprotein. In addition to chemical evidence, the first **electron microscope** pictures of TMV were produced by researchers in Germany. The pictures showed the crystals to have a distinct rod-like shape. For his work in crystallizing the tobacco mosaic virus, Stanley shared the 1946 Nobel prize in chemistry with John Howard Northrop and James Sumner.

During World War II, Stanley was asked to participate in efforts to prevent viral diseases, and he joined the Office of Scientific Research and Development in Washington D.C. Here, he worked on the problem of finding a vaccine effective against viral influenza. Such a substance would change the virus so that the body's **immune system** could build up defenses without causing the disease. Using fertilized hen eggs as a source, he proceeded to grow, isolate, and purify the virus. After many attempts, he discovered that formaldehyde, the chemical used as a biological preservative, would inactivate the virus but still induce the body to produce antibodies. The first flu vaccine was tested and found to be remarkably effective against viral influenza. For his work in developing large-scale methods of preparing vaccines, he was awarded the Presidential Certificate of Merit in 1948.

In 1948, Stanley moved to the University of California in Berkeley, where he became director of a new virology laboratory and chair of the department of biochemistry. In five years, Stanley assembled an impressive team of scientists and technicians who reopened the study of **plant viruses** and began an intensive effort to characterize large, biologically important

molecules. In 1955 Heinz Fraenkel-Conrat, a protein chemist, and R. C. Williams, an electron microscopist, took TMV apart and reassembled the viral **RNA**, thus proving that RNA was the infectious component. In addition, their work indicated that the protein component of TMV served only as a protective cover. Other workers in the virus laboratory succeeded in isolating and crystallizing the virus responsible for polio, and in 1960, Stanley led a group that determined the complete amino acid sequence of TMV protein. In the early 1960s, Stanley became interested in a possible link between viruses and cancer.

Stanley was an advocate of academic freedom. In the 1950s, when his university was embroiled in the politics of McCarthyism, members of the faculty were asked to sign oaths of loyalty to the United States. Although Stanley signed the oath of loyalty, he publicly defended those who chose not to, and his actions led to court decisions which eventually invalidated the requirement.

Stanley received many awards, including the Alder Prize from Harvard University in 1938, the Nichols Medal of the American Chemical Society in 1946, and the Scientific Achievement Award of the American Medical Association in 1966. He held honorary doctorates from many colleges and universities. He was a prolific author of more than 150 publications and he co-edited a three volume compendium entitled *The Viruses*. By lecturing, writing, and appearing on television he helped bring important scientific issues before the public. He served on many boards and commissions, including the National Institute of Health, the **World Health Organization**, and the National Cancer Institute.

Stanley married Marian Staples Jay on June 25, 1929. The two met at the University of Illinois, when they both were graduate students in chemistry. They co-authored a scientific paper together with Adams, which was published the same year they were married. The Stanleys had three daughters and one son. While attending a conference on biochemistry in Spain, Stanley died from a heart attack at the age of 66.

See also History of immunology; History of microbiology; Viral genetics; Viral vectors in gene therapy; Virology; Virus replication; Viruses and responses to viral infection

STAPHYLOCOCCI AND STAPHYLOCOCCI INFECTIONS

Staphylococci are a group of Gram-positive **bacteria** that are members of the genus *Staphylococcus*. Several infections are caused by staphylococci. In particular, infections associated with methicillin-resistant *Staphylococcus aureus* are an increasing problem in hospitals.

The name staphyloccus is derived from Greek (staphyle—a bunch of grapes). The designation describes the typical grape-like clustered arrangement of staphylococci viewed under a light **microscope**. Staphylococci are divided into two groups based on the presence or absence of the plasma-clotting enzyme called **coagulase**. The coagulase-positive staphylococci consist mainly of *Staphylococcus aureus* and the coagulase-negative group consists primarily of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Because the treatment of infections caused by these bacteria can be different, the coagulase test provides a rapid means of indicating the identity of the bacteria of concern.

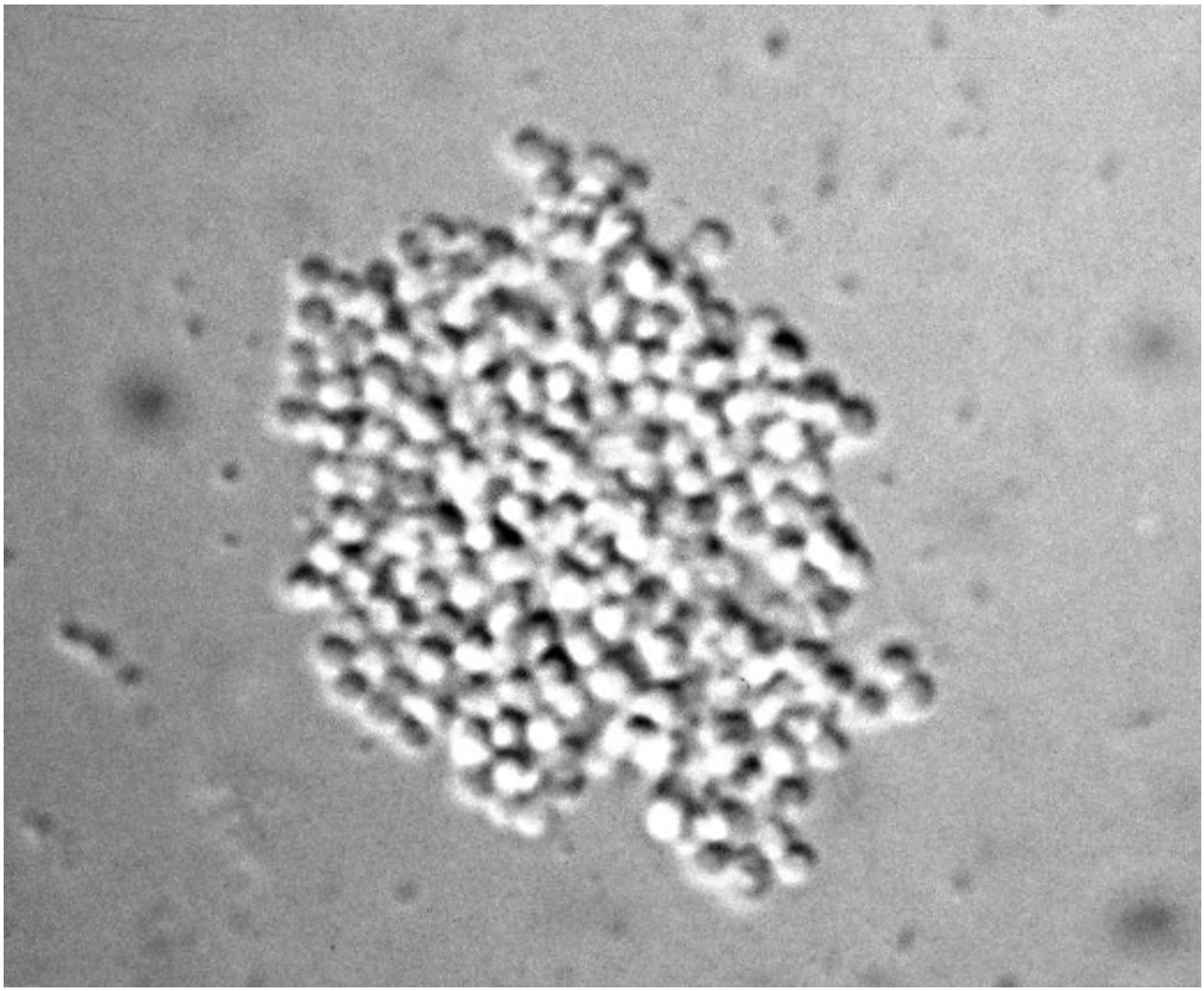
Staphylococci are not capable of movement and do not form spores. They are capable of growth in the presence and absence of oxygen. Furthermore, staphylococci are hardy bacteria, capable of withstanding elevated conditions of temperature, salt concentration, and a wide **pH** range. This hardiness allows them to colonize the surface of the skin and the mucous membranes of many mammals including humans.

Staphylococcus aureus is the cause of a variety of infections in humans. Many are more of an inconvenience than a threat (e.g., skin infection, infection of hair follicles, etc.). However, other infections are serious. One example is a skin infection known as scalded skin syndrome. In newborns and burn victims, scalded skin syndrome can be fatal. Another example is **toxic shock syndrome** that results from the infection of a tampon with a toxin-producing strain (other mechanisms also cause toxic shock syndrome). The latter syndrome can overwhelm the body's defenses, due to the production by the bacteria of what is called a superantigen. This superantigen causes a large proportion of a certain type of immune cells to release a chemical that causes dramatic changes in the physiology of the body.

Staphylococci can also infect wounds. From there, the infection can spread further because some strains of staphylococci produce an arsenal of **enzymes** that dissolve membranes, protein, and degrade both **DNA** and **RNA**. Thus, the bacteria are able to burrow deeper into tissue to evade the host's immune response and antibacterial agents such as **antibiotics**. If the infection spreads to the bloodstream, a widespread **contamination** of the body can result (e.g., **meningitis**, endocarditis, **pneumonia**, bone **inflammation**).

Because staphylococci are resident on the skin of the hands, the bacteria can be easily transferred to objects or people. Within the past few decades the extent to which staphylococci infection of implanted devices is a cause of chronic diseases has become clear. For example, contamination of implanted heart valves and artificial hips joints is now recognized to be the cause of heart damage and infection of the bone.

Additionally, the ready transfer of staphylococci from the skin is an important reason why staphylococci infections are pronounced in settings such as hospitals. *Staphylococcus aureus* is an immense problem as the source of hospital-acquired infections. This is especially true when the strain of bacteria is resistant to the antibiotic methicillin and other common antibiotics. This resistance necessitates more elaborate treatment with more expensive antibiotics. Furthermore, the infection can be more established by the time the **antibiotic resistance** of the bacteria is determined. These so-called methicillin-resistant *Staphylococcus aureus* (MRSA) are resistant to only a few antibiotics currently available. The prevalence of MRSA among all the *Staphylococcus aureus* that is isolated in hospitals in the United States is about 50%. The fear is that the bacteria will acquire resistance to the remaining antibiotics that are currently effective. This fear is



A cluster of Staphylococcus bacteria.

real, since the MRSA is prevalent in an environment (the hospital) where antibiotics are in constant use. Development of a fully resistant strain of *Staphylococcus aureus* would make treatment of MRSA infections extremely difficult, and would severely compromise health care.

Staphylococci are also responsible for the poisoning of foods (e.g., ham, poultry, potato salad, egg salad, custards). The poisoning typically occurs if contaminated food is allowed to remain at a temperature that allows the staphylococci to grow and produce a toxin. Ingestion of the toxin produces an intestinal illness and can affect various organs throughout the body.

The need for more effective prevention and treatment strategies for staphylococcal infections is urgent, given the wide variety of infections that are caused by staphylococci and the looming specter of a completely resistant staphylococcus.

See also Bacteria and bacterial infection; Infection and resistance

STEAM PRESSURE STERILIZER

Steam pressure **sterilization** requires a combination of pressure, high temperatures, and moisture, and serves as one of the most widely used methods for sterilization where these functions will not affect a load. The simplest example of a steam pressure sterilizer is a home pressure cooker, though it is not recommended for accurate sterilization. Its main component is a chamber or vessel in which items for sterilization are sealed and subjected to high temperatures for a specified length of time, known as a cycle.

Steam pressure sterilizer has replaced the term autoclave for all practical purposes, though autoclaving is still

used to describe the process of sterilization by steam. The function of the sterilizer is to kill unwanted **microorganisms** on instruments, in cultures, and even in liquids, because the presence of foreign microbes might negatively affect the outcome of a test, or the purity of a sample. A sterilizer also acts as a test vehicle for industrial products such as plastics that must withstand certain pressures and temperatures.

Larger chambers are typically lined with a metal jacket, creating a pocket to trap pressurized steam. This method preheats the chamber to reduce condensation and cycle time. Surrounding the unit with steam-heated tubes produces the same effect. Steam is then introduced by external piping or, in smaller units, by internal means, and begins to circulate within the chamber. Because steam is lighter than air, it quickly builds enough mass to displace it, forcing interior air and any air-steam mixtures out of a trap or drain.

Most sterilization processes require temperatures higher than that of boiling water (212°F, 100°C), which is not sufficient to kill microorganisms, so pressure is increased within the chamber to increase temperature. For example, at 15 psi the temperature rises to 250°F (121°C). Many clinical applications require a cycle of 20 minutes at this temperature for effective sterilization. Cycle variables can be adjusted to meet the requirements of a given application. The introduction of a vacuum can further increase temperature and reduce cycle time by quickly removing air from the chamber. The process of steam sterilization is kept in check by pressure and temperature gauges, as well as a safety valve that automatically vents the chamber should the internal pressure build beyond the unit's capacity.

See also Infection control; Laboratory techniques in microbiology

STENTOR

Stentor is a genus of protozoan that is found in slow moving or stagnant fresh water. The microorganism is named for a Greek hero in the Trojan War, who was renowned for his loud voice, in an analogous way to the sound of a trumpet rising up over the sound of other instruments. The description is fitting the microorganism because the organism is shaped somewhat like a trumpet, with small end flaring out to form a much larger opening at the other end. The narrow end can elaborate a sticky substance that aids the protozoan in adhering to plants. At the other end, fine hair-like extensions called cilia beat rhythmically to drive food into the gullet of the organism. The various species of stentor tend to be brightly colored. For example, *Stentor coeruleus* is blue in color. Other species are yellow, red, and brown.

Stentor are one of the largest **protozoa** found in water. As a protozoan, *Stentor* is a single cell. Nonetheless, a typical organism can be 2 mm in length, making them visible to the unaided eye, and even larger than some multi-celled organisms such as rotifers. This large size and ubiquity in pond water has made the organism a favorite tool for school science classes, particularly as a learning tool for the use of the light **microscope**. In particular, the various external and internal features are very apparent under the special type of microscopic illumination called phase contrast. Use of other forms of microscopic illumination, such as bright field, dark field, oblique, and Rheinberg illumination, can each reveal features that together comprise a detailed informational picture of the protozoan. Thus, examination of stentor allows a student to experiment with different forms of light microscopic illumination and to directly compare the effects of each type of illumination of the same sample.

Another feature evident in *Stentor* is known as a contractile vacuole. The vacuole functions to collect and cycle back to the outside of *Stentor* the water that flows in to balance the higher salt concentration inside the protozoan. Careful observation of the individual protozoa usually allows detection of full and collapsed vacuoles.

For the student, fall is a good time to observe *Stentor.* Leaves that have fallen into the water decay and support the growth of large numbers of **bacteria**. These, in turn, support the growth of large numbers of stentor.

See also Microscope and microscopy; Water pollution and purification

STERILIZATION

Sterilization is a term that refers to the complete killing or elimination of living organisms in the sample being treated. Sterilization is absolute. After the treatment the sample is either devoid of life, or the possibility of life (as from the subsequent germination and growth of bacterial spores), or it is not.

There are four widely used means of sterilization. Standard sterilization processes utilize heat, radiation, chemicals, or the direct removal of the **microorganisms**.

The most widely practiced method of sterilization is the use of heat. There are a number of different means by which heat can be applied to a sample. The choice of which method of delivery depends on a number of factors including the type of sample. As an example, when bacterial spores are present the heating conditions must be sufficient to kill even these dormant forms of the **bacteria**.

A common type of heat sterilization that is used many types each day in a microbiology laboratory is known as incineration. Microorganisms are burned by exposing them to an open flame of propane. "Flaming" of inoculating needles and the tops of laboratory glassware before and after sampling are examples of incineration.

Another form of heat sterilization is boiling. Drinking water can be sterilized with respect to potentially harmful microorganisms such as ***Escherichia coli*** by heating the water to a temperature of 212°F (100°C) for five minutes. However, the dormant cyst form of the protozoan *Giardia lamblia* that can be present in drinking water, can survive this period of boiling. To ensure complete sterility, the 212°F (100°C) temperature must be maintained for 30 minutes. Even then, some bacterial spores, such as those of *Bacillus* or *Clostridium* can survive. To guarantee sterilization, fluids must be boiled for an

extended time or intermittent boiling can be done, wherein at least three—and up to 30—periods of boiling are interspersed with time to allow the fluid to cool.

Steam heat (moist heat) sterilization is performed on a daily basis in the microbiology laboratory. The pressure cooker called an autoclave is the typical means of steam heat sterilization. Autoclaving for 15 minutes at 15 pounds of pressure produces a temperature of 250°F (121°C), sufficient to kill bacterial spores. Indeed, part of a quality control regiment for a laboratory should include a regular inclusion of commercially available bacterial spores with the load being sterilized. The spores can then be added to a liquid growth medium and growth should not occur.

Pasteurization is employed to sterilize fluids such as milk without compromising the nutritional or flavor qualities of the fluid.

The final form of heat sterilization is known as dry heat sterilization. Essentially this involves the use of an oven to heat dry objects and materials to a temperature of 320–338°F (160–170°C) for two hours. Glassware is often sterilized in this way.

Some samples cannot be sterilized by the use of heat. Devices that contain rubber gaskets and plastic surfaces are often troublesome. Heat sterilization can deform these materials or make them brittle. Fortunately, other means of sterilization exist.

Chemicals or gas can sterilize objects. Ethylene oxide gas is toxic to many microorganisms. Its use requires a special gas chamber, because the vapors are also noxious to humans. Chemicals that can be used to kill microorganisms include formaldehyde and glutaraldehyde. Ethanol is an effective sterilant of laboratory work surfaces. However, the exposure of the surface to ethanol must be long enough to kill the adherent microorganisms, otherwise survivors may develop resistance to the sterilant.

Another means of sterilization utilizes radiation. Irradiation of foods is becoming a more acceptable means of sterilizing the surface of foods (e.g., poultry). Ultraviolet radiation acts by breaking up the genetic material of microorganisms. The damage is usually too severe to be repaired. The sole known exception is the **radiation-resistant bacteria** of the genus *Deinococcus*.

The final method of sterilization involves the physical removal of microorganisms from a fluid. This is done by the use of filters that have extremely small holes in them. Fluid is pumped through the filter, and all but water molecules are excluded from passage. Filters—now in routine use in the treatment of drinking water—can be designed to filter out very small microorganisms, including many **viruses**.

See also Bacterial growth and division; Bacteriocidal, bacteriostatic; Laboratory techniques in microbiology

STREP THROAT

Streptococcal sore throat, or strep throat as it is more commonly called, is an infection caused by group A *Streptococcus* **bacteria**. The main target of the infection is the mucous membranes lining the pharynx. Sometimes the tonsils are also infected (tonsillitis). If left untreated, the infection can develop into rheumatic fever or other serious conditions.

Strep throat is a common malady, accounting for 5–10% of all sore throats. Strep throat is most common in school age children. Children under age two are less likely to get the disease. Adults who smoke, are fatigued, or who live in damp, crowded conditions also develop the disease at higher rates than the general population.

The malady is seasonal. Strep throat occurs most frequently from November to April. In these winter months, the disease passes directly from person to person by coughing, sneezing, and close contact. Very occasionally the disease is passed through food, most often when a food handler infected with strep throat accidentally contaminates food by coughing or sneezing.

Once infected with the *Streptococcus,* a painful sore throat develops from one to five days later. The sore throat can be accompanied by fatigue, a fever, chills, headache, muscle aches, swollen lymph glands, and nausea. Young children may complain of abdominal pain. The tonsils look swollen and are bright red with white or yellow patches of pus on them. Sometimes the roof of the mouth is red or has small red spots. Often a person with strep throat has a characteristic odor to their breath.

Others who are infected may display few symptoms. Still others may develop a fine, rough, sunburn-like rash over the face and upper body, and have a fever of 101–104ºF (38–40ºC). The tongue becomes bright red with a flecked, strawberry-like appearance. When a rash develops, this form of strep throat is called scarlet fever. The rash is a reaction to toxins released by the streptococcus bacteria. Scarlet fever is essentially treated the same way. The rash disappears in about five days. One to three weeks later, patches of skin may peel off, as might occur with a sunburn.

Strep throat can be self-limiting. Symptoms often subside in four or five days. However, in some cases untreated strep throat can cause rheumatic fever. This is a serious illness, although it occurs rarely. The most recent outbreak appeared in the United States in the mid-1980s. Rheumatic fever occurs most often in children between the ages of five and 15, and may have a genetic component, because susceptibility seems to run in families. Although the strep throat that causes rheumatic fever is contagious, rheumatic fever itself is not.

Rheumatic fever begins one to six weeks after an untreated streptococcal infection. The joints, especially the wrists, elbows, knees, and ankles become red, sore, and swollen. The infected person develops a high fever, and possibly a rapid heartbeat when lying down, paleness, shortness of breath, and fluid retention. A red rash over the trunk may come and go for weeks or months. An acute attack of rheumatic fever lasts about three months. Rheumatic fever can cause permanent damage to the heart and heart valves. It can be prevented by promptly treating streptococcal infections with **antibiotics**. It does not occur if all the *Streptococcus* bacteria are killed within the first 10–12 days after infection.

In the 1990s, outbreaks of a virulent strain of group A *Streptococcus* were reported to cause a toxic-shock-like illness and a severe invasive infection called necrotizing fasciitis, which destroys skin and muscle tissue. Although these diseases are caused by group A *Streptococcus*, they rarely begin with strep throat. Usually the *Streptococcus* bacteria enter the body through a skin wound. These complications are rare. However, since the death rate in necrotizing fasciitis is 30–50%, prompt medical attention for any streptococcal infection is prudent.

The *Streptococcus* bacteria are susceptible to antibiotics such as **penicillin**. However, in some 10% of infections, penicillin is ineffective. Then, other antibiotics are used, including amoxicillin, clindamycin, or a cephalosporin.

See also Bacteria and bacterial infection; Streptococci and streptococcal infections

STREPTOCOCCAL ANTIBODY TESTS

Species of Gram positive **bacteria** from the genus *Streptococcus* are capable of causing infections in humans. There are several disease-causing strains of **streptococci**. These strains have been categorized into groups (A, B, C, D, and G), according to their behavior, chemistry, and appearance.

Each group causes specific types of infections and symptoms. For example, group A streptococci are the most virulent species for humans and are the cause of "strep throat," tonsillitis, wound and **skin infections**, blood infections (septicemia), scarlet fever, **pneumonia**, rheumatic fever, Sydenham's chorea (formerly called St. Vitus' dance), and glomerulonephritis.

While the symptoms affected individuals experience may be suggestive of a streptococcal infection, a diagnosis must be confirmed by testing. The most accurate common procedure is to take a sample from the infected area for **culture**, a means whereby the bacteria of interest can be grown and isolated using various synthetic laboratory growth media. This process can take weeks. A more rapid indication of the presence of streptococci can be obtained through the detection of antibodies that have been produced in response to the infecting bacteria. The antibody-based tests can alert the physician to the potential presence of living infectious streptococci.

The presence of streptococci can be detected using antibody-based assays. Three streptococcal **antibody** tests that are used most often are known as the antistreptolysin O titer (ASO), the antideoxyribonuclease-B titer (anti-Dnase-B, or ADB), and the streptozyme test.

The antistreptolysin O titer determines whether an infection with the group A Streptococcus has precluded the development of post-infection complications. The term titer refers to the amount of antibody. Thus, this test is quantitative. That is, the amount of specific antibody in the sample can be deduced. In an infection the amount of antibody will rise, as the **immune system** responds to the invading bacteria. These complications include scarlet fever, rheumatic fever, or a kidney disease termed glomerulonephritis.

The ASO titer is used to demonstrate the body's reaction to an infection caused by group A beta-hemolytic streptococci. The beta-hemolytic designation refers to a reaction produced by the bacteria when grown in the presence of red blood cells. Bacteria of this group are particularly important in suspected cases of acute rheumatic fever (ARF) or acute glomerulonephritis. Group A streptococci produce the enzyme streptolysin O, which can destroy (lyse) red blood cells. Because streptolysin O is antigenic (contains a protein foreign to the body), the body reacts by producing antistreptolysin O (ASO), which is a neutralizing antibody. ASO appears in the blood serum one week to one month after the onset of a strep infection. A high titer (high levels of ASO) is not specific for any type of poststreptococcal disease, but it does indicate if a streptococcal infection is or has been present.

Tests conducted after therapy starts can reveal if an active infection was in progress. This will be evident by a decreasing antibody titer over time, as more and more of the streptococci are killed.

The anti-DNase-B test likewise detects groups A beta-hemolytic Streptococcus. This test is often done at the same time as the ASO titer. This done as the Dnase-based test can produce results that are more variable than those produced by the ASO test. This blanket coverage typically detects some 95% of previous strep infections are detected. If both tests are repeatedly negative, the likelihood is that the patient's symptoms are not caused by a poststreptococcal disease.

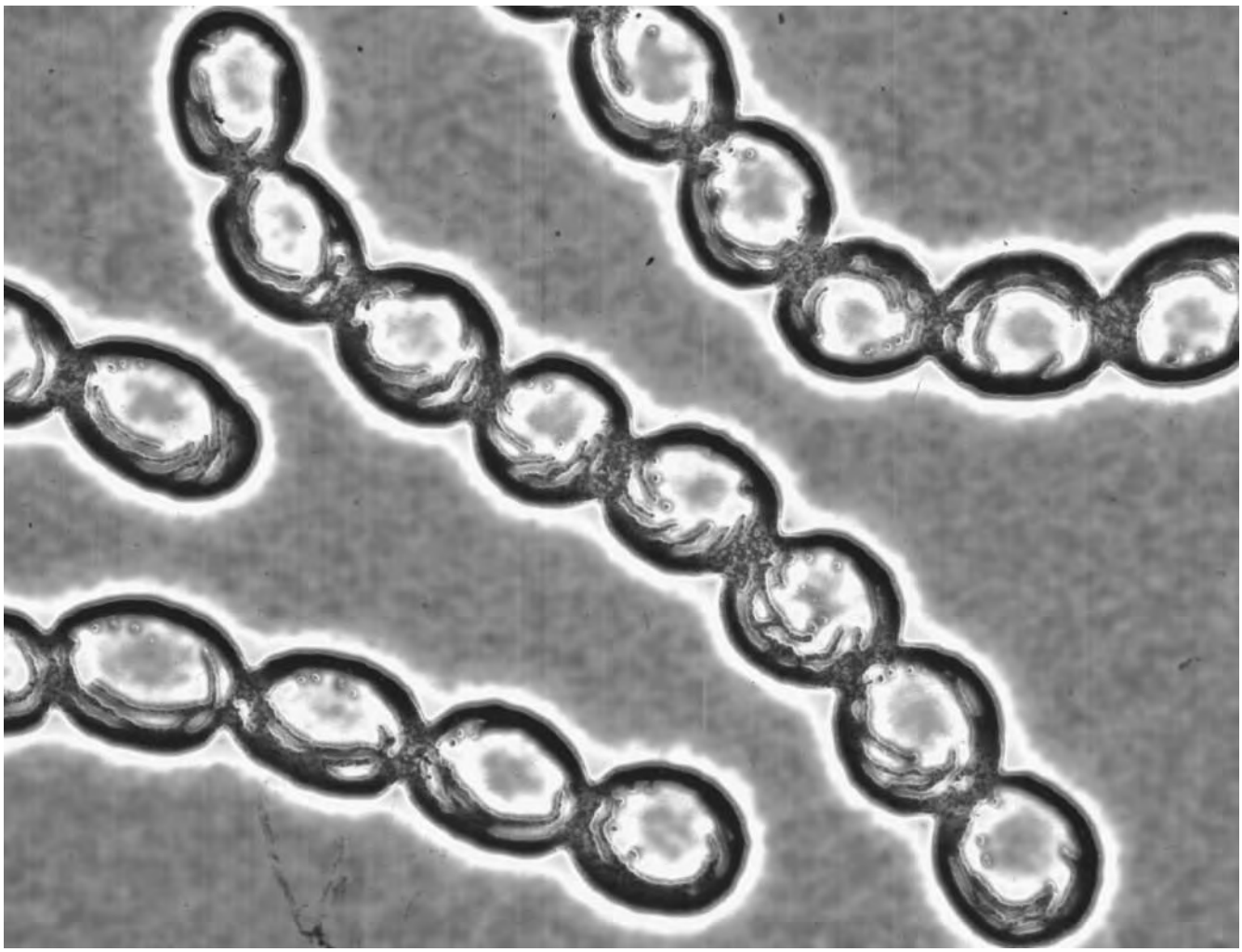
The final antibody-based test is a screening test. That is, the test is somewhat broader in scope than the other tests. The streptozyme test is often used as a screening test for antibodies to the streptococcal antigens NADase, DNase, streptokinase, streptolysin O, and hyaluronidase. This test is most useful in evaluating suspected poststreptococcal disease following infection with *Streptococcus pyogenes*, such as rheumatic fever.

The streptozyme assay has certain advantages over the other two tests. It can detect several antibodies in a single assay, is quick and easy to perform, and is unaffected by factors that can produce false-positives in the ASO test. However, the assay does have some disadvantages. While it detects different antibodies, it does not determine which one has been detected, and it is not as sensitive in children as in adults.

See also Antibody and antigen; Antibody formation and kinetics; Bacteria and bacterial infection

STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS

Streptococci are spherical, Gram positive **bacteria**. Commonly they are referred to as strep bacteria. Streptococci are normal residents on the skin and mucous surfaces on or inside humans. However, when strep bacteria normally found on the skin or in the intestines, mouth, nose, reproductive tract, or urinary tract invade other parts of the body—via a cut or abrasion—and contaminate blood or tissue, infection can be the result.



Chains of *Streptococcus pyogenes*.

Numerous strains of strep bacteria have been identified. Those streptococci from groups A, B, C, D, and G are most likely to cause disease. While some of these infections do not produce symptoms, and the infected person can become a carrier of the disease-causing bacteria, other strep infections can be fatal.

Primary strep infections invade healthy tissue, and most often affect the throat. Secondary strep infections invade tissue already weakened by injury or illness. They frequently affect the bones, ears, eyes, joints, or intestines. Both primary and secondary strep infections can travel from affected tissues to lymph glands, enter the bloodstream, and spread throughout the body.

Group A streptococci contains those strep bacteria that are most apt to be associated with serious illness. Between 10,000 and 15,000 infections attributable to group A streptococci occur in the United States every year. Most are mild inflammations of the throat or skin, the environments where the bacteria are normally found. However, other infections can be deadly.

One example of a serious infection is known as necrotizing fasciitis (which is also referred in the popular press as flesh-eating disease). The disease results from the invasion of host tissue cells by the bacteria. There, shielded from the immune responses of the host, toxic bacterial products cause the destruction of muscle tissue and fat. The infection is able to quickly spread outward from the point of origin. Unless intervention is undertaken quickly, which includes antibiotic therapy and, in severe cases where a limb is involved, amputation of the affected limb, the infection can be fatal.

A second example of a serious group A streptococcal infection manifests **toxic shock syndrome**.

Another division of streptococci is known as group B. Infections caused by group B streptococci most often affects pregnant women, infants, the elderly, and chronically ill adults. Group B was designated in the 1970s. The intervening years have revealed group B streptococci to be the primary cause of life-threatening illness and death in newborns. The bacteria reside in the reproductive tract of a quarter of all pregnant women. Only a small percentage of these women develop inva-

sive infection. However, about half of those who are infected will transmit the bacteria to their babies during delivery.

In the United States, about 12,000 newborns will be infected each year. Of these, about 8,000 develop early-onset infection within hours or days of birth. Complications include **inflammation** of the membranes covering the brain and spinal cord (**meningitis**), **pneumonia**, and blood infection (sepsis). In other infants, meningitis will develop in the first three months of life.

The streptococci in group D are a common cause of wound infections in hospital patients. As well, group D streptococci are associated with abnormal growth of tissue in the gastrointestinal tract, urinary tract infection, and infections of the womb in women who have just given birth.

Another group of the streptococci that is of concern to human health is group G. Normally present on the skin, in the mouth and throat, and in the intestines and genital tract, group G streptococci can cause opportunistic infections in people whose immune systems are compromised by disease, therapy, or neglect. Candidates for infection include severe alcoholics, those with cancer, diabetes mellitus, and rheumatoid arthritis. The bacteria of this group can cause a variety of infections, including infection of the bloodstream (bacteremia), inflammation of the connective tissue structure surrounding a joint (bursitis), infection of various regions of the heart and heart valves (endocarditis), meningitis, inflammation of bone and bone marrow (osteomyelitis), and the inflammation of the lining of the abdomen (peritonitis).

The conventional treatment for streptococcal infections is the administration of **antibiotics**. Many strains of strep are still susceptible to **penicillin**. However, strains of *Streptococcus pneumonia* that are resistant to multiple antibiotics are a problem in hospitals world-wide.

Prevention of infection involves keeping wounds clean and good hygienic practices, such as frequent hand washing, especially before eating and after using the bathroom.

See also Bacteria and bacterial infection

STREPTOMYCIN • *see* ANTIBIOTICS

SULFA DRUGS

Sulfa drugs, developed in the 1930s, were the first medications effective against bacterial disease. They appeared as the first "miracle drugs" at a time when death from bacterial infections such as **pneumonia** and blood poisoning were common.

In 1932, German physician and biomedical researcher Gerhard Domagk was working on a project for the German industrial giant I. G. Farbenindustrie to test industrial chemicals for medical utility. One of the chemicals was a dye called Prontosil, or sulfamidochrysoidine. Domagk hypothesized that since the dye worked by binding to the proteins in fabric and leather, it might also bind to the proteins in **bacteria**, thus inhibiting their action. Experiments on laboratory animals infected with streptococcus were promising, and were soon followed by successful clinical tests.

In 1936, Prontosil was successfully used against puerperal sepsis, or "childbed fever," which was killing thousands of mothers every year. It was also shown to be effective against **meningitis**, pneumonia, and streptococcal infections.

Meanwhile, scientists at the Pasteur Institute in Paris discovered that upon ingestion, the dye molecule was cleaved in two, and that the active part, sulfanilamide, was just as effective on its own. This was important because the smaller molecule was not covered by Farben's patent on Prontosil, and was also less expensive to produce.

There followed a rush by pharmaceutical companies in the United States and Europe to develop sulfa drugs of their own. Among the most effective were sulfapyridine for pneumonia, sulfathiazole against pneumonia and staphylococcus, sulfaguanadine to treat **dysentery**, and sulfadiazine, which worked against pneumonia, strep and staph. Domagk was awarded the Nobel Prize in Medicine in 1939, but World War II prevented him from receiving his medal until 1947.

Investigating the action of the sulfa drugs led to an important new understanding of the action of pharmaceuticals. Sulfanilamides compete with the action of para-aminobenzoic acid (PABA), which bacteria use to produce folic acid. Without folic acid, the bacteria cannot synthesize **DNA**. This is an example of a common drug mechanism called antagonism. A structurally similar molecule can work against a substance necessary to the **metabolism** of a microorganism (or involved in some other disease process) by competitively binding to the same enzyme and thus blocking its action.

A tragic episode involving a sulfa drug was also important in medical history because of its effect on United States law. In 1937, the S. E. Massengill Company released a sulfa medication in liquid form. Unfortunately, a toxic solvent (the medium suspending the sulfa medication) was used, and more than 100 people died. The next year, the Federal Food, Drug and Cosmetics Act was passed, requiring that new drugs be tested for safety.

The ability to fight dysentery and other bacterial diseases with sulfa drugs was important to soldiers in World War II. However, too much sulfa was bad for the kidneys, and by the end of the war, **penicillin** and other newly developed **antibiotics** with fewer side effects became increasingly available and preferred in treatment. In addition, many bacterial strains have developed resistance against sulfa drugs in the decades since they were developed, which has also limited their usefulness. Regardless, they are still effective against some infections, including **leprosy**, and are often used in developing nations because of their low cost.

See also Antibiotic resistance, tests for; Antibiotics; Bacteria and bacterial infection; Bioterrorism, protective measures; History of the development of antibiotics; History of public health; Infection and resistance; Penicillin; Streptococci and streptococcal infections

SULFUR CYCLE IN MICROORGANISMS

Sulfur is a key constituent of certain amino acids, proteins, and other biochemicals of both **eukaryotes** and prokaryotes. For example, sulfur is a component of an enzyme called coenzyme A, which is vital for **respiration** of plant and animal cells.

Plants are not able to directly use elemental sulfur. Instead, they rely on the ability of certain types of **bacteria** to convert elemental sulfur to another form. Bacteria that are known as **chemoautotrophic bacteria** can combine sulfur with water and oxygen to produce hydrogen sulfate. Plants are able to incorporate the sulfate compound into proteins.

Bacteria can participate in the reduction of sulfur, in which the sulfur compounds act as an electron receptor, or in the oxidation of sulfur, in which an electron is removed from the sulfur compound.

Hydrogen sulfide, a gas that has the characteristic smell of rotten eggs, is toxic to air-requiring plant and animal tissue. However, the gas can be utilized by oxygen-requiring bacteria such as *Thiothrix* and *Beggiatoa*, and by the anaerobic purple sulfur bacteria. These bacteria utilize the hydrogen sulfide and carbon dioxide to produce elemental sulfur.

Sulfur can occur in many chemically reduced mineral forms, or sulfides, in association with many metals. The most common metal sulfides in the environment are iron sulfides (called pyrites when they occur as cubic crystals), but all heavy metals can occur in this mineral form. Whenever metal sulfides are exposed to an oxygen-rich environment, certain bacteria begin to oxidize the sulfide, generating sulfate as a product, and tapping energy from the process that is used to sustain their own growth and reproduction. This autotrophic process is called chemosynthesis, and the bacteria involved are named *Thiobacillus thiooxidans*. When a large quantity of sulfide is oxidized in this way, an enormous amount of acidity is associated with the sulfate product. Indeed, *Thiobacillus prosperus* has an optimum **pH** of between pH=1 and pH=4, and *Thiobacillus ferroxidans* has an optimum pH range of between pH=2 and pH=4.

Some species of the genus *Thiobacillus*, including *Thiobacillus thiooxidans* and *Thiobacillus ferroxidans* are able to process elemental sulfur and iron sulfate, respectively.

Within the past several decades, the existence of bacteria that utilize sulfur at **hydrothermal vents** deep within the ocean has been chronicled. These bacteria form the basis of the entire complex ecosystem that springs up, in the total absence of light, around the sulfur-rich emission form the vents. Some of the bacteria live in symbiosis with the so-called tubeworms that thrive in this ecosystem. The worms provide protection and an incoming supply of nutrients to the bacteria. In turn, the bacteria metabolize the sulfur to forms usable to the worms. The discovery of the bacterial basis of this undersea ecosystem greatly increased human awareness of the microbial diversity on Earth.

See also Biogeochemical cycles; Economic uses and benefits of microorganisms

SUPERFUND • *see* BIOREMEDIATION

SWINE FEVER

Swine fever is a viral disease that afflicts swine. The disease is also known as hog cholera. A related form of the disease is called African swine fever.

The virus that causes swine fever is a member of the family Flaviviridae and the genus *Pestivirus*. A virus causes African swine fever from the family Iridovirisae. The virus itself is designated *Asfarviridae*, a name derived from "African Swine Fever and Related Viruses." The virus is so far the sole member of the newly created genus *Asfivirus*.

The two **viruses** are quite different from one another in structure and behavior. Yet, the diseases they cause are very similar with respect to their transmission and the symptoms of infection. Both viruses can be easily passed from an infected pig to a healthy pig. Contact can be direct or via body secretions or feces. The resulting infection can be mild or more severe. Also a long lasting form of infection can result. The more severe form of the infection results in a very high fever that can lead to convulsions. Often the skin appears discolored and pigs will huddle together. Death usually results a week or two weeks after the appearance of symptoms. The chronic form of the infection displays similar but less severe symptoms. The symptoms can persist for months before the swine succumbs. Finally, an infection can display few if any symptoms. However, this mild bout of the disease can caused reduce number of live births.

Swine that survive the infections can be life-long carriers of the viruses.

Distinction of swine fever from African swine fever is only possible by the direct examination of the viruses. The examinations typically involve the isolation of the virus in an appropriate cell **culture** and the use of fluorescent-labeled antibodies and the **enzyme-linked immunosorbant assay** (**ELISA**).

Both viruses are easily spread from hog to hog. Pigs and related animals such as wild boar are the only natural reservoirs of *Pestivirus*. *Asfivirus* can reside in species such as ticks. The viruses can also be accidentally carried from an infected swine to a susceptible swine via humans, animals, and birds. This is in part due to the environmental persistence of the viruses. For example, *Pestivirus* is able to survive cold conditions, and so can survive in a refrigerated carcass during transport. As well, the virus is able to survive some forms of meat processing (e.g., curing and smoking). However, *Pestivirus* is susceptible to various disinfectants (e.g., sodium hydroxide, Formalin, various detergents).

Pestivirus infects the blood and virtually all body fluids of an infected animal. Furthermore, the animal can excrete the virus for months.

The adverse effects to the health of the swine, and to their economic value, has made the eradication of swine fevers a priority in many countries around the world. In the United States, for example, a concerted effort by State and Federal governments and industry over almost two decades has virtu-

ally eliminated the disease in the country. However, vigilance is necessary to maintain this record. In Great Britain, where swine fever had been eliminated by 1966, it reappeared in 2000.

Such is not the case around the world. In many countries, swine fever remains a problem. Belgium and France experienced heavy economic losses in 1997, for example. African swine fever is a major problem affecting swine in countries such s Gambia, Ghana, and Madagascar, and there have also been outbreaks in more northern countries (e.g., Italy in 1999 and Portugal in 2000).

In countries such as the United States, swine entering the country are quarantined for 90 days to ensure that the swine do not harbor the virus that has yet to be evident as an infection.

Currently there is no treatment for either swine fever, save slaughter of the infected animals. In this regard, swine fever is similar to foot and mouth disease that afflicts cattle and sheep. The use of a **vaccine** consisting of weakened but living virus has been an effective preventative measure for swine fever. However, unless the **vaccination** involves the total swine population in the target region, the prevention of infection will not be absolute.

See also Virology

SYNCHRONOUS GROWTH

Synchronous growth is the growth of **bacteria** such that all the bacteria are at the same stage in their growth cycle (e.g., exponential phase, stationary phase). Because the same cellular reactions occur simultaneously throughout the bacterial population, synchronous growth permits the detection of events not normally detectable in a single cell or in a population consisting of bacteria in various stages of growth.

In a normal batch **culture** of fluid, or on an **agar** plate, bacteria in the population exhibit a range of sizes, ages, and growth rates. In contrast, the bacteria in a synchronized culture are virtually identical in terms of these parameters.

Synchronized growth is imposed in the laboratory. A population of bacteria can be filtered to obtain bacteria of a certain size range. Usually, the filter that is used has very small holes. All but the smallest bacteria in a population are excluded from passing through the filter. Because the smallest bacteria are frequently the youngest bacteria, the filtering method selects for a population comprised of bacteria that usually have just completed a division event. When the bacteria are suspended in fresh growth medium the population will subsequently grow and then divide at the same rate.

Bacteria of the same size can also be recovered using special techniques of centrifugation, where the bacteria in the fluid that is spinning around in a centrifuge are separated on the basis of their different densities. The smallest bacteria will have the lowest density and so will move furthest down the centrifuge tube.

Another method of obtaining a synchronous bacterial population involves the manipulation of some environmental factor that the bacteria depend on for growth. Typically, the factor is a nutrient that the bacteria cannot manufacture, and so is required to be present in the medium. In the alternative, an agent (e.g., an antibiotic) can be added that does not kill the bacteria but rather halts their growth at a certain point. Again, once the bacteria are added to fresh medium, the growth of all the bacteria will recommence from the point of blockage in the **cell cycle**.

Synchronous growth can only be maintained for a few rounds of growth and division. Ultimately, the inherent randomness of bacterial population growth again dominates. In other words, not all the bacteria will continue to divide at exactly and differences in size and other attributes will once again appear in the population. For those few generations, however, much useful information can be extracted from a synchronously growing population.

See also Bacterial growth and division; Laboratory techniques in microbiology

SYPHILIS

Syphilis is a chronic, degenerative, sexually transmitted disease caused by the bacterium *Treponema pallidum*. Although modern treatments now control the disease, its incidence remains high worldwide, making it a global **public health** concern. Spread by sexual contact, syphilis begins as a small, hard, painless swelling, called a primary (or Hunter's) chancre. The disease is very contagious in the early stages. The initial sore will usually pass away in about eight weeks, but the disease will then spread through the body and lodge in the lymph nodes, causing a skin rash to appear in two to four months along with fever and headaches. This second stage can last two to six weeks. After a latent period, which can extend for years, the disease can appear in various bodily organs and it can be spread to others.

The earliest records of syphilis are those of Spanish physician Rodrigo Ruiz de Isla, who wrote that he treated syphilis patients in Barcelona in 1493. He further claimed that the soldiers of explorer Christopher Columbus contracted the disease in the Caribbean and brought it back to Europe in 1492. However, others challenge this position. Some medical historians believe that syphilis has been present from ancient times but was often mislabeled or misdiagnosed. Italian physician and writer Girolamo Fracastoro gave the disease its name in his poem "Syphilis sive morbus Gallicus" (Syphilis or the French Disease), published in 1530, during the height of a European epidemic. However, for centuries, the disease was called pox or the great pox. At that time, the treatment was mercury, used in vapor baths, as an ointment, or taken orally. The mercury increased the flow of saliva and phlegm to wash out the poisons, but it also caused discomfort, such as loss of hair and teeth, abdominal pains, and mouth sores. Through the centuries, a milder form of the disease evolved and often became confused with **gonorrhea**. In 1767, physician John Hunter infected himself with fluid from a patient who had gonorrhea to prove these were two different diseases. Unknown to Hunter,

the patient also had syphilis. Hunter developed the sore indicative of syphilis that now bears his name.

The distinction between the two diseases was made clear in 1879, when German bacteriologist Albert Neisser isolated the bacterium responsible for gonorrhea. In 1903, Russian biologist Elie Metchnikoff and French scientist Pierre-Paul-Emile Roux demonstrated that syphilis could be transmitted to monkeys and then studied in the laboratory. They also showed that mercury ointment was an effective treatment in the early stages. Two years later, German zoologist Fritz Schaudinn and his assistant Erich Hoffmann discovered the bacterium responsible for syphilis, the spiral-shaped spirochete *Treponema pallidum*. The following year, German physician August von Wassermann (1866–1925) developed the first diagnostic test for syphilis based on new findings in **immunology**. The test involved checking for the syphilis **antibody** in a sample of blood. One drawback was that the test would take two days to complete.

In 1904, German research physician **Paul Ehrlich** began focusing on a safe, effective treatment for syphilis. Ehrlich had spent many years studying the effect of dyes on biological tissues and treatments for tropical diseases. His work in the emerging field of immunology earned him a Nobel Prize in 1908. Ehrlich began working with the arsenic-based compound atoxyl as a possible treatment for syphilis. Japanese bacteriologist Sahachiro Hata came to study syphilis with Ehrlich. Hata tested hundreds of derivatives of atoxyl and finally found one that worked, number 606. Ehrlich called it Salvarsan. Following clinical trials, in 1911 Ehrlich and Hata announced the drug was an effective cure for syphilis. The drug attacked the disease germs but did not harm healthy cells; thus, Salvarsan ushered in the new field of **chemotherapy**. Ehrlich went on to develop two safer forms of the drug, including neosalvarsan in 1912 and sodium salvarsan in 1913.

Penicillin came into widespread use in treating bacterial diseases during World War II. It was first used to against syphilis in 1943 by New York physician John F. Mahoney, and it remains the treatment of choice today. Other **antibiotics** are also effective. Meanwhile, Russian-American researcher Reuben Leon Kahn (1887–1979) developed a modified test for syphilis in 1923 that took only a few minutes to complete. Another test was developed by researchers William A. Hinton (1883–1959) and J. A. V. Davies. Today fluorescent antibody tests are used for detection. Although there is no inoculation for syphilis, the disease can be controlled through education, safe sexual practices, and proper medical treatment.

See also Sexually transmitted diseases

T

T-CELL LEUKEMIA VIRUS • *see* HUMAN T-CELL LEUKEMIA VIRUS (HTLV)

T CELLS OR T-LYMPHOCYTES

When a vertebrate encounters substances that are capable of causing it harm, a protective system known as the **immune system** comes into play. This system is a network of many different organs that work together to recognize foreign substances and destroy them. The immune system can respond to the presence of a disease-causing agent (pathogen) in two ways. Immune cells called the **B cells** can produce soluble proteins (antibodies) that can accurately target and kill the pathogen. This branch of **immunity** is called "humoral immunity." In cell-mediated immunity, immune cells known as the T cells produce special chemicals that can specifically isolate the pathogen and destroy it.

The T cells and the B cells together are called the lymphocytes. The precursors of both types of cells are produced in the bone marrow. While the B cells mature in the bone marrow, the precursors to the T cells leave the bone marrow and mature in the thymus. Hence the name, "T cells" for thymus-derived cells.

The role of the T cells in the immune response is to specifically recognize the pathogens that enter the body and to destroy them. They do this either by directly killing the cells that have been invaded by the pathogen, or by releasing soluble chemicals called **cytokines**, which can stimulate other killer cells specifically capable of destroying the pathogen.

During the process of maturation in the thymus, the T cells are taught to discriminate between self (an individual's own body cells) and non-self (foreign cells or pathogens). The immature T cells, while developing and differentiating in the thymus, are exposed to the different thymic cells. Only those T cells that are self-tolerant, that is to say, they will not interact with the molecules normally expressed on the different body cells are allowed to leave the thymus. Cells that react with the body's own proteins are eliminated by a process known as "clonal deletion." The process of clonal deletion ensures that the mature T cells, which circulate in the blood, will not interact with or destroy an individual's own tissues and organs. The mature T cells can be divided into two subsets, the T-4 cells (that have the accessory molecule CD4), or the T-8 (that have CD8 as the accessory molecule).

There are millions of T cells in the body. Each T cell has a unique protein structure on its surface known as the T cell receptor (TCR), which is made before the cells ever encounter an **antigen**. The TCR can recognize and bind only to a molecule that has a complementary structure. It is kind of like a lock-and key arrangement. Each TCR has a unique binding site that can attach to a specific portion of the antigen called the epitope. As stated before, the binding depends on the complementarity of the surface of the receptor and the surface of the epitope. If the binding surfaces are complementary, and the T cells can effectively bind to the antigen, then it can set into motion the immunological cascade which eventually results in the destruction of the pathogen.

The first step in the destruction of the pathogen is the activation of the T cells. Once the T-lymphocytes are activated, they are stimulated to multiply. Special cytokines called interleukins that are produced by the T-4 lymphocytes mediate this proliferation. It results in the production of thousands of identical cells, all of which are specific for the original antigen. This process of clonal proliferation ensures that enough cells are produced to mount a successful immune response. The large clone of identical lymphocytes then differentiates into different cells that can destroy the original antigen.

The T-8 lymphocytes differentiate into cytotoxic T-lymphocytes (CTLs) that can destroy the body cells that have the original antigenic epitope on its surface, e.g., bacterial infected cells, viral infected cells, and tumor cells. Some of the T lymphocytes become memory cells. These cells are capable of remembering the original antigen. If the individual is exposed to the same **bacteria** or virus again, these memory

Bison grazing near hot springs. Bacteria growing in hot springs are the source of taq.

cells will initiate a rapid and strong immune response against it. This is the reason why the body develops a permanent immunity after an infectious disease.

Certain other cells known as the T-8 suppressor cells play a role in turning off the immune response once the antigen has been removed. This is one of the ways by which the immune response is regulated.

See also Bacteria and bacterial infection; Immune stimulation, as a vaccine; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunochemistry; Immunological analysis techniques; Immunology, nutritional aspects; Viruses and responses to viral infection

TAQ ENZYME

A taq enzyme is a bacterial enzyme that functions in the manufacture of **deoxyribonucleic acid** (**DNA**). The ability of the enzyme to function at higher temperatures than other similarly functioning bacterial **enzymes** has made it valuable in the **polymerase chain reaction**.

The moniker taq denotes the origin of the enzyme. The enzyme is produced by a bacterium known as *Thermus aquaticus*. This bacterium was discovered by Thomas Brock in the mid 1970s in the nearly boiling waters of Mushroom pool, a hot spring in Yellowstone National Park

Taq is a DNA polymerase. The enzyme manufactures a strand of DNA that is complimentary to a single strand of DNA. All **bacteria** possess DNA polymerase. The reason that the taq polymerase has become so significant to biotechnological processes is because of the resistance of the enzyme to heat. A **molecular biology** technique known as the polymerase chain reaction relies upon the exposure of DNA to heat in order to separate the two strands of the double helix. Taq can then use both of the single strands as templates for the manufacture of two new strands of DNA. To perform this function, the polymerase is able to recognize the particular building block, or nucleotide, on the DNA single strand and then position a nucleotide that is the complimentary match to the particular target. Binding of the two nucleotides occurs. The polymerase can then move on to the next nucleotide and the process is repeated. When the DNA mixture is allowed to cool the matching strands link together forming two double stranded helices of DNA. If this process is repeated many times, a huge number of copies of the target region of DNA can be manufactured. The heat resistance of taq allows the enzyme to function in the temperature conditions that keep the DNA strands apart from each other. The DNA polymerase

from other bacteria, for example from the bacterium ***Escherichia coli***, do not function nearly as efficiently in the polymerase chain reaction as the taq polymerase of *Thermus aquaticus*.

Since the discovery of taq enzyme and the development of the polymerase chain reaction, the importance of the enzyme to molecular biology research and commercial applications of **biotechnology** have soared. Taq polymerase is widely used in the molecular diagnosis of maladies and in forensics ("DNA fingerprinting"). These and other applications of taq have spawned an industry worth hundreds of millions of dollars annually.

See also DNA (Deoxyribonucleic acid); DNA hybridization; Extremophiles; Molecular biology and molecular genetics; PCR

TATUM, EDWARD LAWRIE (1909-1975)

American biochemist

Edward Lawrie Tatum's experiments with simple organisms demonstrated that cell processes can be studied as chemical reactions and that such reactions are governed by genes. With George Beadle, he offered conclusive proof in 1941 that each biochemical reaction in the cell is controlled via a catalyzing enzyme by a specific **gene**. The "one gene-one enzyme" theory changed the face of biology and gave it a new chemical expression. Tatum, collaborating with **Joshua Lederberg**, demonstrated in 1947 that **bacteria** reproduce sexually, thus introducing a new experimental organism into the study of **molecular genetics**. Spurred by Tatum's discoveries, other scientists worked to understand the precise chemical nature of the unit of heredity called the gene. This study culminated in 1953, with the description by James Watson and **Francis Crick** of the structure of **DNA**. Tatum's use of **microorganisms** and laboratory **mutations** for the study of biochemical genetics led directly to the **biotechnology** revolution of the 1980s. Tatum and Beadle shared the 1958 Nobel Prize in physiology or medicine with Joshua Lederberg for ushering in the new era of modern biology.

Tatum was born in Boulder, Colorado, to Arthur Lawrie Tatum and Mabel Webb Tatum. He was the first of three children. Tatum's father held two degrees, an M.D. and a Ph.D. in pharmacology. Edward's mother was one of the first women to graduate from the University of Colorado. As a boy, Edward played the French horn and trumpet; his interest in music lasted his whole life.

Tatum earned his A.B. degree in chemistry from the University of Wisconsin in 1931, where his father had moved the family in order to accept as position as professor in 1931. In 1932, Tatum earned his master's degree in microbiology. Two years later, in 1934, he received a Ph.D. in **biochemistry** for a dissertation on the cellular biochemistry and nutritional needs of a bacterium. Understanding the biochemistry of microorganisms such as bacteria, **yeast**, and molds would persist at the heart of Tatum's career.

In 1937, Tatum was appointed a research associate at Stanford University in the department of biological sciences. There he embarked on the *Drosophila* (fruit fly) project with geneticist George Beadle, successfully determining that kynurenine was the enzyme responsible for the fly's eye color, and that it was controlled by one of the eye-pigment genes. This and other observations led them to postulate several theories about the relationship between genes and biochemical reactions. Yet, the scientists realized that *Drosophila* was not an ideal experimental organism on which to continue their work.

Tatum and Beadle began searching for a suitable organism. After some discussion and a review of the literature, they settled on a pink **mold** that commonly grows on bread known as *Neurospora crassa*. The advantages of working with ***Neurospora*** were many: it reproduced very quickly, its nutritional needs and biochemical pathways were already well known, and it had the useful capability of being able to reproduce both sexually and asexually. This last characteristic made it possible to grow cultures that were genetically identical, and also to grow cultures that were the result of a cross between two different parent strains. With *Neurospora,* Tatum and Beadle were ready to demonstrate the effect of genes on cellular biochemistry.

The two scientists began their *Neurospora* experiments in March 1941. At that time, scientists spoke of "genes" as the units of heredity without fully understanding what a gene might look like or how it might act. Although they realized that genes were located on the **chromosomes**, they didn't know what the chemical nature of such a substance might be. An understanding of DNA (**deoxyribonucleic acid**, the molecule of heredity) was still 12 years in the future. Nevertheless, geneticists in the 1940s had accepted Gregor Mendel's work with inheritance patterns in pea plants. Mendel's theory, rediscovered by three independent investigators in 1900, states that an inherited characteristic is determined by the combination of two hereditary units (genes), one each contributed by the parental cells. A dominant gene is expressed even when it is carried by only one of a pair of chromosomes, while a recessive gene must be carried by both chromosomes to be expressed. With *Drosophila*, Tatum and Beadle had taken genetic **mutants**—flies that inherited a variant form of eye color—and tried to work out the biochemical steps that led to the abnormal eye color. Their goal was to identify the variant enzyme, presumably governed by a single gene that controlled the variant eye color. This proved technically difficult, and as luck would have it, another lab announced the discovery of kynurenine's role before theirs did. With the *Neurospora* experiments, they set out to prove their one gene-one enzyme theory another way.

The two investigators began with biochemical processes they understood well: the nutritional needs of *Neurospora*. By exposing cultures of *Neurospora* to x rays, they would cause genetic damage to some bread mold genes. If their theory was right, and genes did indeed control biochemical reactions, the genetically damaged strains of mold would show changes in their ability to produce nutrients. If supplied with some basic salts and sugars, normal *Neurospora*

can make all the amino acids and vitamins it needs to live except for one (biotin).

This is exactly what happened. In the course of their research, the men created, with x-ray bombardment, a number of mutated strains that each lacked the ability to produce a particular amino acid or vitamin. The first strain they identified, after 299 attempts to determine its mutation, lacked the ability to make vitamin B_6. By crossing this strain with a normal strain, the offspring inherited the defect as a recessive gene according to the inheritance patterns described by Mendel. This proved that the mutation was a genetic defect, capable of being passed to successive generations and causing the same nutritional mutation in those offspring. The x-ray bombardment had altered the gene governing the enzyme needed to promote the production of vitamin B_6.

This simple experiment heralded the dawn of a new age in biology, one in which molecular genetics would soon dominate. Nearly 40 years later, on Tatum's death, Joshua Lederberg told the *New York Times* that this experiment "gave impetus and morale" to scientists who strived to understand how genes directed the processes of life. For the first time, biologists believed that it might be possible to understand and quantify the living cell's processes.

Tatum and Beadle were not the first, as it turned out, to postulate the one gene-one enzyme theory. By 1942, the work of English physician Archibald Garrod, long ignored, had been rediscovered. In his study of people suffering from a particular inherited enzyme deficiency, Garrod had noticed the disease seemed to be inherited as a Mendelian recessive. This suggested a link between one gene and one enzyme. Yet Tatum and Beadle were the first to offer extensive experimental evidence for the theory. Their use of laboratory methods, like x rays, to create genetic mutations also introduced a powerful tool for future experiments in biochemical genetics.

During World War II, the methods Tatum and Beadle had developed in their work with pink bread mold were used to produce large amounts of **penicillin**, another mold. In 1945, at the end of the war, Tatum accepted an appointment at Yale University as an associate professor of botany with the promise of establishing a program of biochemical microbiology within that department. In 1946. Tatum did indeed create a new program at Yale and became a professor of microbiology. In work begun at Stanford and continued at Yale, he demonstrated that the one gene-one enzyme theory applied to yeast and bacteria as well as molds.

In a second fruitful collaboration, Tatum began working with Joshua Lederberg in March 1946. Lederberg, a Columbia University medical student 15 years younger than Tatum, was at Yale during a break in the medical school curriculum. Tatum and Lederberg began studying the bacterium ***Escherichia coli***. At that time, it was believed that *E. coli* reproduced asexually. The two scientists proved otherwise. When cultures of two different mutant bacteria were mixed, a third strain, one showing characteristics taken from each parent, resulted. This discovery of biparental inheritance in bacteria, which Tatum called genetic **recombination**, provided geneticists with a new experimental organism. Again, Tatum's methods had altered the practices of experimental biology. Lederberg never returned to medical school, earning instead a Ph.D. from Yale.

In 1948 Tatum returned to Stanford as professor of biology. A new administration at Stanford and its department of biology had invited him to return in a position suited to his expertise and ability. While in this second residence at Stanford, Tatum helped establish the department of biochemistry. In 1956, he became a professor of biochemistry and head of the department. Increasingly, Tatum's talents were devoted to promoting science at an administrative level. He was instrumental in relocating the Stanford Medical School from San Francisco to the university campus in Palo Alto. In that year Tatum also was divorced, then remarried in New York City. Tatum left the West coast and took a position at the Rockefeller Institute for Medical Research (now Rockefeller University) in January 1957. There he continued to work through institutional channels to support young scientists, and served on various national committees. Unlike some other administrators, he emphasized nurturing individual investigators rather than specific kinds of projects. His own research continued in efforts to understand the genetics of *Neurospora* and the nucleic acid **metabolism** of mammalian cells in **culture**.

In 1958, together with Beadle and Lederberg, Tatum received the Nobel Prize in physiology or medicine. The Nobel Committee awarded the prize to the three investigators for their work demonstrating that genes regulate the chemical processes of the cell. Tatum and Beadle shared one-half the prize and Lederberg received the other half for work done separately from Tatum. Lederberg later paid tribute to Tatum for his role in Lederberg's decision to study the effects of x-ray-induced mutation. In his Nobel lecture, Tatum predicted that "with real understanding of the roles of heredity and environment, together with the consequent improvement in man's physical capacities and greater freedom from physical disease, will come an improvement in his approach to, and understanding of, sociological and economic problems."

Tatum's second wife, Viola, died in 1974. Tatum married Elsie Bergland later in 1974 and she survived his death the following year, in 1975. Tatum died at his home on East Sixty-third Street in New York City after an extended illness, at age 65.

See also Fungal genetics; Microbial genetics; Molecular biology and molecular genetics; Molecular biology, central dogma of

TECHNOLOGY AND TECHNIQUES IN IDENTIFICATION OF MICROORGANISMS

• *see* GENETIC IDENTIFICATION OF MICROORGANISMS

TEM

• *see* ELECTRON MICROSCOPE, TRANSMISSION AND SCANNING

TERRORISM, USE OF MICROBIOLOGICAL AGENTS

• *see* BIOTERRORISM

TETANUS AND TETANUS IMMUNIZATION

Tetanus is a bacterial disease that affects the nervous system in humans. The disease is caused by the **bacteria** *Clostridium tetani*. This organism, which is a common inhabitant of soil, dust, and manure, can contaminate an abrasion in the skin. Small cuts and pinpoint wounds can be contaminated. Because the organism can survive and grow in the absence of oxygen, deep wounds, such as those caused by puncture with a nail or a deep cut by a knife, are especially susceptible to infections with *Clostridium tetani*. The disease cannot be transmitted from one person to another.

In addition to being able to grow in oxygen-free environments, such as is found in a deep wound, *Clostridium tetani* is able to hibernate in environments such as the soil. This is because the bacteria can convert from an actively growing and dividing state, when conditions are favorable for growth, to a dormant state, when growth conditions are more hostile. Dormancy is achieved by the conversion of the so-called vegetative cell to an endospore. Essentially, an endospore is an armored ball in which the genetic material of the organism can be stored, in a form that resists heat, dryness, and lack of nutrients. When conditions once again become favorable, such as in the nutrient-rich and warm environment of a wound, the dormant bacteria revive and begin to grow and divide once more.

Tetanus is also commonly known as lockjaw, in recognition of the stiffening of the jaw that occurs because of the severe muscle spasms triggered by the infecting bacteria. The muscle paralysis restricts swallowing, and may even lead to death by suffocation. The muscular stiffening of the jaw, along with a headache, are usually the first symptoms of infection. These typically begin about a week after infection has begun. Some people experience symptoms as early as three days or as late as three weeks following the start of an infection. Following the early symptoms, swallowing becomes difficult. Other symptoms include the stiffening of the abdominal muscles, muscle spasms, sweating, and fever.

The muscle contractions can be so severe that, in some cases, they have actually broken bones with which they are associated. Treatment can include drugs to stimulate muscle relaxation, neutralize toxin that has not yet had a chance to react with the nervous system, and the administration of **antibiotics** to fight the **bacterial infection**. In spite of these efforts, three of every 10 people who contract tetanus will die from the effects of the disease. As of 2001, 50–100 cases of tetanus occur each year, usually involving people who either have never taken protective measures against the disease or who have let this protection lapse. In the absence of the protective measures such as **vaccination**, many more people would develop tetanus.

Interestingly, another group who are susceptible to tetanus are heroin addicts who inject themselves with a compound called quinine. This compound is used to dilute the heroin. Available evidence indicates that quinine may actually promote the growth of *Clostridium tetani*, by an as yet unknown mechanism.

For those who survive tetanus, recovery can take months and is not an easy process. Muscle stiffness and weakness may persist.

The molecular basis of the effects of infection by *Clostridium tetani* is a very potent toxin produced and excreted from the bacteria. The toxin is a neurotoxin. That is, the toxin affects neurons that are involved in transmitting signals to and from the brain in order to make possible the myriad of functions of the body. Specifically, in tetanus the neurotoxin blocks the release of neurotransmitters.

Clostridium tetani neurotoxin is composed of two chains of protein that are linked together. An enzyme present in the microorganism cuts these chains apart, which makes the toxin capable of the neurotransmitter inhibitory activity. One of the chains is called tetanospasmin. It binds to the ends of neurons and blocks the transmission of impulses. This blockage results in the characteristic spasms of the infection. The other toxin chain is known as tetanolysin. This chain has a structure that allows it to insert itself into the membrane surrounding the neuron. The inserted protein actually forms a pore, or hole, through the membrane. Molecules can move freely back and forth through the hole, which disrupts the functioning of the membrane.

The devastating effects of tetanus are entirely preventable. Vaccination in childhood, and even in adulthood, can prevent an infection from developing if *Clostridium tetani* should subsequently gain entry to a wound. Indeed, in the United States, laws requiring children to be immunized against tetanus now exist in most states, and all states require children in day care facilities to be immunized against tetanus.

Tetanus vaccination involves the administration of what is called tetanus toxoid. In use since the 1920s, tetanus toxoid is inactivated tetanus toxin. Injection of the toxoid stimulates the production of antibodies that will act to neutralize the active toxin. The toxoid can be given on its own. But typically, the toxoid is administered in combination with vaccines against **diphtheria** and **pertussis** (diphtheria toxoid pertussis, or DTP, **vaccine**). The DTP vaccine is given to children several times (two months after birth, four months, six months, 15 months, and between four and six years of age). Thereafter, a booster injection should be given every 10 years to maintain the **immunity** to tetanus. A lapse in the 10-year cycle of vaccination can leave a person susceptible to infection.

Tetanus toxoid will not provide protection to someone who has already been wounded. There is a substance called tetanus immune globulin that can provide immediate immunity.

The tetanus vaccination can produce side effects, ranging from slight fever and crankiness to severe, but non-lethal convulsions. Very rarely, brain damage has resulted from vaccination. Even though the possibility of the serious side effects is far outweighed by the health risks of foregoing vaccination, controversy exists over the wisdom of tetanus vaccination. Available evidence indicates that tetanus **immunization** is a wise measure.

See also Anaerobes and anaerobic infections

TETRACYCLINES • *see* ANTIBIOTICS

THE INSTITUTE FOR GENOMIC RESEARCH (TIGR)

The Institute for Genomic Research (TIGR) is a non-profit research institute located in Rockville, Maryland. The primary interest of TIGR is the sequencing of the genomes and the subsequent analysis of the sequences in prokaryotic and eukaryotic organisms. J. Craig Venter founded TIGR in 1992 and acted as president until 1998. As of 2002, Venter remained as chairman of the board of trustees for TIGR.

TIGR scientists sequenced the genomes of a number of **viruses**, **bacteria**, archaebacteria, plants, animals, **fungi**, and **protozoa**. The sequences of the bacteria *Haemophilus influenzae* and *Mycoplasma genitalium*, published in 1996, were the first complete bacterial **DNA** sequences ever accomplished. In 1996, the complete sequence of an archaebacteria (*Methanococcus jannaschii*) was published. Since that time, TIGR has sequenced 19 other bacterial genomes. These include the genomes of the bacteria that cause cholera, **tuberculosis**, **meningitis**, **syphilis**, **Lyme disease**, and stomach ulcers. In addition, TIGR sequenced the genome of the protozoan parasite *Plasmodium falciparum*, the cause of **malaria**.

The genesis of TIGR was the automation of the DNA sequencing process. This advance made the idea of large-scale sequencing efforts tangible. At about the same time, Venter was the leader of a section at the National Institute of Neurological Disorders and Stroke. He developed a technique called **shotgun cloning** that could efficiently and rapidly sequence large stretches of DNA. Use of the bacterial artificial **chromosomes** in a sequencing strategy that had been developed by Venter allowed large sections of the human genome to be inserted into the bacterium ***Escherichia coli*** where many copies of the sequences could be produced for sequence analysis. This technique proved to be much faster than the more conventional sequencing technique that was simultaneously being done by the United States government. The technique involved the creation of many overlapping fragments of the DNA, determination of the sequences, and then, using the common sequences present in the overlapping regions, piecing together the fragments to produce the full sequence of a genome. However, the concept was not readily accepted. At the time, the conventional sequencing strategy was to begin sequencing at one end of the genome and progress through to the other end in a linear manner.

In 1992, Venter left the National Institutes of Health and, with the receipt of a 10-year, $70 million grant from a private company, he founded TIGR in order to utilize the shotgun **cloning** philosophy as applied to the large-scale sequencing of genetic information.

Acceptance of Venter's and TIGR's approach to **gene** sequencing came with the 1995 publication of the genome sequence of the bacterium *Haemophilus influenzae*. This represented the first determination of a genome sequence of a living organism.

Another major research trust at TIGR has been the development of software analysis programs that sift through the vast amounts of sequence information in order to identify probable gene sequences. Also, programs are being developed to permit the analysis of these putative genes and the presentation of the structure of the proteins they code for. A technology known as micro-arraying is being refined. In this technique, thousands of genes can be placed onto a support for simultaneous analysis. This and other initiatives hold the promise of greatly increasing the speed of DNA sequencing.

TIGR also gained widespread public notoriety for its involvement in the sequencing of the human genome. Specifically, TIGR's establishment thrust the issue of corporate ownership of genetic information into the forefront of public awareness. Backed by the financing necessary to begin operations, TIGR partnered with an organization called Human Genome Sciences. The latter company had first opportunity to utilize any sequences emerging from TIGR labs. The specter of genetic information, especially that associated with diseases, being controlled by a private interest was, and remains, extremely controversial.

In 1997, TIGR dissolved the partnership with Human Genome Services. Since then, the genetic sequencing efforts have moved more toward the public domain. For example, now all TIGR gene sequences are posted on the organization's web site and the institute spearheads public forums and symposia.

TIGR is now headquartered on a 17-acre facility on the outskirts of Washington, D.C., and the institute is comprised of nearly 200 research staff.

See also Biotechnology; DNA (Deoxyribonucleic acid); Genetic mapping

THEILER, MAX (1899-1972)

South African virologist

Max Theiler (pronounced Tyler) was a leading scientist in the development of the yellow-fever **vaccine**. His early research proved that yellow-fever virus could be transmitted to mice. He later extended this research to show that mice that were given serum from humans or animals that had been previously infected with **yellow fever** developed **immunity** to this disease. From this research, he developed two different vaccines in the 1930s, which were used to control this incurable tropical disease. For his work on the yellow-fever vaccine, Theiler was awarded the Nobel Prize in medicine or physiology in 1951.

Theiler was born on a farm near Pretoria, South Africa, on January 30, 1899, the youngest of four children of Emma (Jegge) and Sir Arnold Theiler, both of whom had emigrated from Switzerland. His father, director of South Africa's veterinary services, pushed him toward a career in medicine. In part to satisfy his father, he enrolled in a two-year premedical program at the University of Cape Town in 1916. In 1919, soon after the conclusion of World War I, he sailed for England, where he pursued further medical training at St. Thomas's Hospital Medical School and the London School of **Hygiene** and Tropical Medicine, two branches of the

University of London. Despite this rigorous training, Theiler never received the M.D. degree because the University of London refused to recognize his two years of training at the University of Cape Town.

Theiler was not enthralled with medicine and had not intended to become a general practitioner. He was frustrated by the ineffectiveness of most medical procedures and the lack of cures for serious illnesses. After finishing his medical training in 1922, the 23-year-old Theiler obtained a position as an assistant in the Department of Tropical Medicine at Harvard Medical School. His early research, highly influenced by the example and writings of American bacteriologist Hans Zinsser, focused on amoebic **dysentery** and rat-bite fever. From there, he developed an interest in the yellow-fever virus.

Yellow fever is a tropical viral disease that causes severe fever, slow pulse, bleeding in the stomach, jaundice, and the notorious symptom, "black vomit." The disease is fatal in 10–15% of cases, the cause of death being complete shutdown of the liver or kidneys. Most people recover completely, after a painful, extended illness, with complete immunity to reinfection. The first known outbreak of yellow fever devastated Mexico in 1648. The last major breakout in the continental United States claimed 435 lives in New Orleans in 1905. Despite the medical advances of the twentieth century, this tropical disease remains incurable. As early as the eighteenth century, mosquitoes were thought to have some relation to yellow fever. Cuban physician Carlos Finlay speculated that mosquitoes were the carriers of this disease in 1881, but his writings were largely ignored by the medical community. Roughly 20 years later, members of America's Yellow Fever Commission, led by Walter Reed, the famous U.S. Army surgeon, concluded that mosquitoes were the medium that spread the disease. In 1901, Reed's group, using humans as research subjects, discovered that yellow fever was caused by a bloodborne virus. Encouraged by these findings, the Rockefeller Foundation launched a world-wide program in 1916 designed to control and eventually eradicate yellow fever.

By the 1920s, yellow fever research shifted away from an all-out war on mosquitoes to attempts to find a vaccine to prevent the spread of the disease. In 1928, researchers discovered that the Rhesus monkey, unlike most other monkeys, could contract yellow fever and could be used for experimentation. Theiler's first big breakthrough was his discovery that mice could be used experimentally in place of the monkey and that they had several practical research advantages.

One unintended research discovery kept Theiler out of his lab and in bed for nearly a week. In the course of his experiments, he accidentally contracted yellow fever from one of his mice, which caused a slight fever and weakness. Theiler was much luckier than some other yellow-fever researchers. Many had succumbed to the disease in the course of their investigations. However, this small bout of yellow fever simply gave Theiler immunity to the disease. In effect, he was the first recipient of a yellow-fever vaccine.

In 1930, Theiler reported his findings on the effectiveness of using mice for yellow fever research in the respected journal *Science*. The initial response was overwhelmingly negative; the Harvard faculty, including Theiler's immediate supervisor, seemed particularly unimpressed. Undaunted, Theiler continued his work, moving from Harvard University, to the Rockefeller Foundation in New York City. Eventually, yellow-fever researchers began to see the logic behind Theiler's use of the mouse and followed his lead. His continued experiments made the mouse the research animal of choice. By passing the yellow-fever virus from mouse to mouse, he was able to shorten the incubation time and increase the virulence of the disease, which enabled research data to be generated more quickly and cheaply. He was now certain that an attenuated live vaccine, one weak enough to cause no harm yet strong enough to generate immunity, could be developed.

In 1931, Theiler developed the mouse-protection test, which involved mixing yellow-fever virus with human blood and injecting the mixture into a mouse. If the mouse survived, then the blood had obviously neutralized the virus, proving that the blood donor was immune to yellow fever (and had most likely developed an immunity by previously contracting the disease). This test was used to conduct the first worldwide survey of the distribution of yellow fever.

A colleague at the Rockefeller Foundation, Dr. Wilbur A. Sawyer, used Theiler's mouse strain, a combination of yellow fever virus and immune serum, to develop a human vaccine. Sawyer is often wrongly credited with inventing the first human yellow-fever vaccine. He simply transferred Theiler's work from the mouse to humans. Ten workers in the Rockefeller labs were inoculated with the mouse strain, with no apparent side effects. The mouse-virus strain was subsequently used by the French government to immunize French colonials in West Africa, a hot spot for yellow fever. This so-called "scratch" vaccine was a combination of infected mouse brain tissue and **cowpox** virus and could be quickly administered by scratching the vaccine into the skin. It was used throughout Africa for nearly 25 years and led to the near total eradication of yellow fever in the major African cities.

While encouraged with the new vaccine, Theiler considered the mouse strain inappropriate for human use. In some cases, the vaccine led to encephalitis in a few recipients and caused less severe side effects, such as headache or nausea, in many others. Theiler believed that a "killed" vaccine, which used a dead virus, wouldn't produce an immune effect, so he and his colleagues set out to find a milder live strain. He began working with the Asibi yellow-fever strain, a form of the virus so powerful that it killed monkeys instantly when injected under the skin. The Asibi strain thrived in a number of media, including chicken embryos. Theiler kept this virus alive for years in tissue cultures, passing it from embryo to embryo, and only occasionally testing the potency of the virus in a living animal. He continued making subcultures of the virus until he reached strain number 176. Then, he tested the strain on two monkeys. Both animals survived and seemed to have acquired a sufficient immunity to yellow fever. In March 1937, after testing this new vaccine on himself and others, Theiler announced that he had developed a new, safer, attenuated vaccine, which he called 17D strain. This new strain was much easier to produce, cheaper, and caused very mild side effects.

From 1940 to 1947, with the financial assistance of the Rockefeller Foundation, more than 28 million 17D-strain vac-

cines were produced, at a cost of approximately two cents per unit, and given away to people in tropical countries and the United States. The vaccine was so effective that the Rockefeller Foundation ended its yellow-fever program in 1949, safe in the knowledge that the disease had been effectively eradicated worldwide and that any subsequent outbreaks could be controlled with the new vaccine. Unfortunately, almost all yellow-fever research ended around this time and few people studied how to cure the disease. For people in tropical climates who live outside of the major urban centers, yellow fever is still a problem. A major outbreak in Ethiopia in 1960–1962 caused 30,000 deaths. The **World Health Organization** still uses Theiler's 17D vaccine and had mounted efforts to inoculate people in remote areas.

The success of the vaccine brought Theiler recognition both in the U.S. Over the next ten years, he received the Chalmer's Medal of the Royal Society of Tropical Medicine and Hygiene (1939), the Lasker Award of the American Public Health Association, and the Flattery Medal of Harvard University (1945).

In 1951, Theiler received the Nobel Prize in medicine or physiology "for his discoveries concerning yellow fever and how to combat it."

After developing the yellow-fever vaccine, Theiler turned his attention to other **viruses**, including some unusual and rare diseases, such as Bwamba fever and Rift Valley fever. His other, less exotic research focused on polio and led to his discovery of a polio-like infection in mice known as encephalomyelitis or Theiler's disease. In 1964, he retired from the Rockefeller Foundation, having achieved the rank of associate director for medical and natural sciences and director of the Virus Laboratories. In that same year, he accepted a position as professor of **epidemiology** and microbiology at Yale University in New Haven, Connecticut. He retired from Yale in 1967.

Theiler married in 1938 and had one daughter. Theiler died on August 11, 1972, at the age of 73.

See also Epidemics, viral; Epidemiology, tracking diseases with technology; History of immunology; History of public health; Immune stimulation, as a vaccine; Viruses and responses to viral infection; Zoonoses

THERMAL DEATH

Thermal death is the death of a population of **microorganisms** due to exposure to an elevated temperature.

The nature of the thermal death varies depending on the source of the heat. The heat of an open flame incinerates the microorganisms. The dry heat of an oven causes the complete removal of water, which is lethal for biological structures. In contrast, the moist heat delivered by a sterilizer such as an autoclave causes the proteins in the sample to coagulate in a way that is analogous to the coagulation of the proteins of an egg to form the familiar cooked egg white.

The coagulation of proteins by heat is a drastic alteration in the three-dimensional shape of these protein molecules. Typically, the alteration is irreversible and renders a protein incapable of proper function.

Thermal death also involves the destruction of the membranes surrounding microorganisms such as **bacteria**. The high temperatures can cause the phospholipid constituents of the membrane to dissolve and thus destroy the membrane structure. Finally, the high heat will also cause the destruction of the nucleic acid of the target microorganism. In the case of double-stranded **DNA**, the heat will result in the disassociation of the two DNA strands.

Thermal death can be related to time. A term known as the thermal death time is defined as the time required to kill a population of the target microorganism in a water-based solution at a given temperature. The thermal death time of microorganisms can vary, depending on the thermal tolerance of the microbes. For example, thermophilic bacteria such as *Thermophilus aquaticus* that can tolerate high temperatures will have a thermal death time that is longer than the more heat-sensitive bacterium ***Escherichia coli***.

Another aspect or measure of thermal death is termed the thermal death point. This is defined as the lowest temperature that will completely kill a population of a target microorganism within 10 minutes. This aspect of thermal death is useful in purifying water via boiling. Whereas *Escherichia coli* populations will be readily killed within 10 minutes at 212°F (100°C), spores of bacteria such as *Bacillus subtilus* and *Clostridium perfringens* will have a higher thermal death point, because a higher temperature is required to kill spores within 10 minutes.

Exact temperatures and times are usually used in calculating thermal death variables because terms such as "boiling" are not precise. For example, the boiling point of water (i.e., the temperature of boiling water) depends upon pressure. As altitude above sea level increases, the boiling temperature of water (H_2O) lowers.

See also Laboratory techniques in microbiology; Sterilization

THERMOTOLERANT BACTERIA • *see* EXTREMOPHILES

THIN SECTIONS AND THIN SECTIONING • *see* ELECTRON MICROSCOPIC EXAMINATION OF MICROORGANISMS

THRUSH

Thrush, or oropharyngeal **candidiasis**, is an infection of the mouth and throat caused by the fungus *Candida*, a genus of **yeast**. This microorganism is naturally present on the skin and mucous membranes, but overgrowth can cause disease. Candidiasis is not considered communicable because the microorganism is ubiquitous (common and widespread).

Symptoms of thrush include cottage cheese-like white patches in the mouth and throat, with raw areas underneath.

Esophageal involvement may result in difficulty in swallowing, nausea, vomiting, and chest pain. Candidiasis is confirmed by **culture** from a swab of the infected tissue.

Proliferation of *Candida* is most often the result of a weakened **immune system**. Candidiasis is one of the most common and visible opportunistic infections that strike people with **AIDS**, **chemotherapy** patients, and other immunocompromised individuals. Many AIDS patients have been first diagnosed after they, or their dentists, noticed a thrush infection. In individuals with normal immune systems, candidiasis may be associated with antibiotic use. Infants, diabetics, smokers, and denture wearers are particularly susceptible to thrush.

In addition to causing thrush, *Candida* may affect the gastrointestinal tract or genitals. The microorganism may also enter the bloodstream, either via surgery or catheterization, or through damage to the skin or mucosa. If the immune system is unable to clear the fungus from the bloodstream, a dangerous systemic infection may occur, resulting in endocarditis, **meningitis**, or other serious problems.

Antifungal medications such as fluconazole and clotrimazole are generally effective in treating candidiasis. However, drug-resistant strains of *Candida* are becoming increasingly prevalent, and recurrence is common. This situation is driving research into new therapies and potential vaccines.

See also Bacteria and bacterial infection; Fungal genetics; Fungi; Fungicide; Immunodeficiency; Immunosuppressant drugs; Infection and resistance; Infection control; Microbial flora of the oral cavity, dental caries; Yeast genetics; Yeast, infectious

TIGR

• *see* THE INSTITUTE FOR GENOMIC RESEARCH (TIGR)

TMV

• *see* TOBACCO MOSAIC VIRUS (TMV)

TOBACCO MOSAIC VIRUS (TMV)

Tobacco mosaic virus (TMV), also known as tobamovirus, is a rod-shaped virus with **ribonucleic acid** (**RNA**) surrounded by a coat of protein that causes mosaic-like symptoms in plants. Mosaic-like symptoms are characterized by mottled patches of green or yellow color on the leaves of infected plants. The virus causes abnormal cellular function that usually does not kill the plant but stunts growth. Infected plants may have brittle stems, abnormally small, curled leaves, and unripened fruit.

Tobacco mosaic virus is capable of infecting many kinds of plants, not just tobacco plants. TMV is spread through small wounds caused by handling, insects, or broken leaf hairs that result from leaves rubbing together. The virus attaches to the cell wall, injects its RNA into the host cell, and forces the host cell to produce new viral RNA and proteins. Finally, the viral RNA and proteins assemble into new **viruses** and infect other cells by passing through small openings called plasmodesmata that connect adjacent plant cells. This process allows the virus to take over metabolic processes without killing cells.

Tobacco mosaic virus is highly infectious and can survive for many years in dried plant parts. Currently, there is no **vaccine** to protect plants from TMV, nor is there any treatment to eliminate the virus from infected plants. However, seeds that carry TMV externally can be treated by acid extraction or trisodium phosphate and seeds that carry the virus internally can receive dry heat treatments.

The discovery of viruses came about in the late 1800's when scientists were looking for the **bacteria** responsible for damaging tobacco plants. During one experiment in 1892, Russian biologist Dimitri Ivanovsky concluded that the disease in tobacco plants could not be caused by bacteria because it passed through a fine-pored filter that is too small for bacteria to pass through. In 1933, American biologist Wendell Stanley of the Rockefeller Institute discovered that the infectious agent formed crystals when purified. The purified extract continued to cause infection when applied to healthy tobacco plants and therefore, could not be a living organism. Soon after, scientists were able to break down the virus into its constituent parts. Today, it is known that the infectious agent that causes the disease in tobacco plants is a virus, not bacteria.

See also Genetic regulation of prokaryotic cells; Plant viruses; Viral genetics; Virus replication

TOXIC SHOCK SYNDROME

Toxic shock syndrome is an illness caused by the bacterium *Staphylococcus aureus*. The syndrome was first recognized in the 1970s when women who were wearing a "superabsorbant" tampon for their menstrual flow developed the illness. The majority of cases occur with this population. Less frequently, toxic shock syndrome can occur in females who do not use tampons, as well as in males.

The symptoms of toxic shock syndrome are caused by a toxin that is produced by *Staphylococcus aureus*. The exact nature of the association of the **bacterial growth** in superabsorbant tampons and the production of the toxin remains unclear. Whatever the exact cause, the cell-density behavior of other **bacteria** lends support to the suggestion that toxin production is triggered by the accumulation of large numbers of the bacteria. In the syndrome occurring in males or women who do not use tampons, there is usually a staphylococcal infection present in the body.

The symptoms of toxic shock syndrome include a sudden high fever, nausea with vomiting, diarrhea, headache, aches all over the body, dizziness and disorientation, a sunburn-like rash on the palms of the hands and the soles of the feet, and a decrease in blood pressure. The latter can send a victim into shock and can result in death. Those who recover may have permanent kidney and liver damage.

These symptoms are produced by the particular toxin that is released by the bacteria. The toxin can enter the bloodstream and move throughout the body. The toxin has been called a "superantigen" because of its potent stimulation of cells of the

immune system. The immune cells release a compound called cytokine. Normally, only a small proportion of the immune cells are releasing cytokine. But the massive cytokine release that occurs in response to the staphylococcal toxin produces the myriad of physiological changes in the body.

Treatment of toxic shock syndrome depends on the prompt recognition of the symptoms and their potential severity. Immediate administration of **antibiotics** is essential.

The number of cases of toxic shock syndrome has been reduced since the suspect superabsorbant tampons were withdrawn from the marketplace.

See also Bacteria and bacterial infection; Enterotoxin and exotoxin; Immune system

TOXOPLASMOSIS

Toxoplasmosis is an infectious disease caused by the protozoan *Toxoplasma gondii*. The infection results from a parasitic association with a human host.

Cats are the primary carrier of the protozoan *Toxoplasma gondii*. In the United States, approximately 30% of cats are at some time infected by *Toxoplasma gondii*. Cattle, sheep, or other livestock can also excrete a form of the protozoan known as an oocyst. Although oocysts are not capable of producing an infection, they are important because they act to preserve the infectious capability of the protozoan during exposure to inhospitable environments. In this capacity they are analogous to the bacterial spore. Oocysts are often capable of resuscitation into the infectious form after prolonged periods of exposure to adverse environments.

Humans can also become infected by eating fruits and vegetables that have themselves become contaminated when irrigated with untreated water contaminated with oocyte-containing feces.

Humans typically contract toxoplasmosis by eating cyst-contaminated raw or undercooked meat, vegetables, or milk products. The protozoan can also be spread from litter boxes or a sandbox soiled with cat feces. In all cases, the agent that is ingested can be the inactive oocyst or the actively growing and infectious egg form of the parasite.

In the human host, the parasite is able to grow and divide. This causes the symptoms of the infection.

Symptoms of toxoplasmosis include a sporadic and reoccurring fever, muscle pain, and a general feeling of malaise. Upon recovery, a life-long **immunity** is conferred. In some people, the disease can become chronic and cause an **inflammation** of the eyes, called retinochoroiditis, that can lead to blindness, severe yellowing of the skin and whites of the eyes (jaundice), easy bruising, and convulsions. As well inflammation of the brain (encephalitis), one-sided weakness or numbness, mood and personality changes, vision disturbances, muscle spasms, and severe headaches can result.

Person to person transmission is not frequent. Such transmission occurs only during pregnancy. Some six out of 1,000 women contract toxoplasmosis during pregnancy. Nearly half of these infections are passed on to the fetus. Congenital toxoplasmosis afflicts approximately 3,300 newborns in the United States each year. In such children, symptoms may be severe and quickly fatal, or may not appear until several months, or even years, after birth.

As for many other microbial diseases, the observance of good **hygiene** (including appropriate hand washing protocols) is a key means of preventing toxoplasmosis.

See also Immunodeficiency diseases; Protozoa; Zoonoses

TRACKING DISEASES WITH TECHNOLOGY

• *see* EPIDEMIOLOGY, TRACKING DISEASES WITH TECHNOLOGY

TRANSCRIPTION

Transcription is defined as the transfer of genetic information from **deoxyribonucleic acid** (**DNA**) to **ribonucleic acid** (**RNA**). The process of transcription in prokaryotic cells (e.g., **bacteria**) differs from the process in eukaryotic cells (cells with a true **nucleus**) but the underlying result of both transcription processes is the same, which is to provide a template for the formation of proteins.

The use of DNA as a blueprint to manufacture RNA begins with an enzyme called RNA polymerase. The enzyme is guided to a certain region on the DNA, called the promoter, by association with molecules known as sigma factors. There are many promoters on DNA, located just before a region of DNA that codes for a protein. The promoter serves to position the RNA polymerase so that transcription of the full coding region is accomplished.

Once the polymerase has bound to a promoter, the sigma factors detach and can serve another polymerase. The attached polymerase then begins to move along the DNA, unwinding the two strands of DNA that are linked together and using the sequence on one of the strands as the blueprint for RNA manufacture. The strand from which RNA is made is known as the template or the antisense strand, while the other strand to which it is complimentary is called the sense or the coding strand.

As the polymerase moves along the DNA, the strands link back together behind the polymerase. The effect is somewhat similar to a zipper with a bulge, where the two links of the zipper have come apart. The bulging region can move along the zipper, with separation and reannealing of the strands occurring continuously with time. The promoter can accommodate the binding of another polymerase as soon as the region is free. Thus, the same stretch of DNA can be undergoing several rounds of transcription at the same end, with polymerase molecules positioned all along the DNA.

The RNA that is produced is known as messenger RNA (or mRNA). The species derives its name from its function. It is the tangible form of the message that is encoded in the DNA. The mRNA in turn functions as a template for the next step in the genetic process, that of **translation**. In translation the mRNA information is used to manufacture protein.

Transcription of a strand of the DNA double helix by DNA polymerase to form messenger RNA.

Termination of transcription occurs when the RNA polymerase reaches a signal on the DNA template strand that signals the polymerase to stop and to end the association with the DNA.

Some **microorganisms** have variations on the basic transcription mechanism. For example, in **yeast** cells the mRNA can be "capped" by the addition of specialized pieces of nucleic acid called telomeres to either end of the transcribed molecule. The telomeres function to extend the life of the mRNA and provide a signal of the importance of the information contained within.

The intricate and coordinated transcription process in bacteria is also a rapid process. For example, measurements in ***Escherichia coli*** have established that the RNA polymerase moves along the DNA at a speed of 50 nucleotides per second.

See also Bacterial artificial chromosome; Genetic regulation of prokaryotic cells

TRANSDUCTION

Transduction is defined as the transfer of genetic information between cells using a type of virus particle called a **bacteriophage**. The virus contains genetic material from one cell, which is introduced into the other cell upon virus infection of the second cell. Transduction does not, therefore, require cell to cell contact and is resistant to **enzymes** that can degrade **DNA**.

Bacteriophage can infect the recipient cell and commandeer the host's replication machinery to produce more copies of itself. This is referred to as the lytic cycle. Alternatively, the phage genetic material can integrate into the host DNA where it can replicate undetected along with the host until such time as an activation signal stimulates the production of new virus particles. This is referred to as the lysogenic cycle. Transduction relies on the establishment of the lysogenic cycle, with the bacterial DNA becoming incorporated into the recipient cell chromosome along with the phage DNA. This means of transferring **bacteria** DNA has been exploited for genetic research with bacteria like *Escherichia coli, Salmonella typhimurium,* and *Bacillus subtilis,* which are specifically targeted by certain types of bacteriophage.

There are two types of transduction: generalized transduction and specialized transduction. In generalized transduction, the packaging of bacterial DNA inside the phage particle that subsequently infects another bacterial cell occurs due to error. The error rate is about one phage particle in 1,000. Experimental **mutants** of phage have been engineered where the error rate is higher. Once the bacterial DNA has been injected inside the second bacterium, there is approximately a 10percent change that the DNA will be stably incorporated into the chromosome of the recipient. A successful integration changes the **genotype and phenotype** of the recipient, which is called a transductant. A transductant will arise for about every 10^6 phage particles that contain bacterial DNA.

Specialized transduction utilizes specialized phage, in which some of the phage genetic material has been replaced by other genetic material, typically the bacterial chromosome. All of the phage particles carry the same portion of the bacterial chromosome. The phage can introduce their DNA into the recipient bacterium as above or via **recombination** between the chromosomal DNA carried by the phage and the chromosome itself.

Transduction has proved to be a useful means of transferring genetic traits from one bacterial cell to another.

See also Bacterial ultrastructure; Bacteriophage and bacteriophage typing; Molecular biology and molecular genetics; Viral genetics; Viral vectors in gene therapy; Virus replication; Viruses and responses to viral infection

TRANSFORMATION

Transformation is a process in which exogenous **DNA** is taken up by a (recipient) cell, sphaeroplast, or protoplast. In order to take up DNA, the cells must be competent. Competence is a state of bacterial cells during which the usually rigid cell wall can transport a relatively large DNA macromolecule. This is a highly unusual process, for **bacteria** normally lack the ability to transport macromolecules across the rigid cell wall and through the cyotplasmic membrane. Several bacteria, such as *Bacillus,*

Haemophilis, Neisseria, and *Streptococcus,* possess natural competence because their cells do not require special treatment to take up DNA. This process is transient and occurs only in special growth phases, typically toward the end of log phase.

The demonstration of DNA transformation was a landmark in the history of genetics. In 1944, Oswald Avery, Colin MacLeod, and **Maclyn McCarty** conducted famous *Streptococcus pneumoniae* transformation experiments. Bacterial **pneumonia** is caused by the S strain of *S. pneumoniae*. The S strain synthesizes a slimy capsule around each cell. The capsule is composed of a polysaccharide that protects the bacterium from the immune response of the infected animal and enables the bacterium to cause the disease. The colonies of the S strain appear smooth because of the capsule formation. The strain that does not synthesize the polysaccharide, hence does not have the capsule, is called R strain because the surface of the colonies looks rough. The R strain does not cause the disease. When heat-killed S strain was mixed with live R strain, cultured, and spread on to a solid medium, a few S strain colonies appeared. When S cell extract was treated with RNase or proteinase and mixed with the live R strain, R colonies and a few S colonies appeared. When the S strain cell extract was treated with DNase and mixed with live R strain, there were only R strain colonies growing on the **agar** plates. These experiments proved fundamentally that DNA is the genetic material that carries genes.

Transformation is widely used in DNA manipulation in **molecular biology**. For most bacteria that do not possess natural competency, special treatment, such as calcium chloride treatment, can render the cells competent. This is one of the most important techniques for introducing recombinant DNA molecules into bacteria and **yeast** cells in genetic engineering.

See also Cell membrane transport; Microbial genetics

TRANSGENICS

The term transgenics refers to the process of transferring genetic information from one organism to another. By introducing new genetic material into a cell or individual, a transgenic organism is created that has new characteristics it did not have before. The genes transferred from one organism or cell to another are called transgenes. The development of biotechnological techniques has led to the creation of transgenic **bacteria**, plants, and animals that have great advantages over their natural counterparts and sometimes act as living machines to create pharmaceutical therapies for the treatment of disease. Despite the advantages of transgenics, some people have great concern regarding the use of transgenic plants as food, and with the possibility of transgenic organisms escaping into the environment where they may upset ecosystem balance.

Except for retroviruses that utilize **ribonucleic acid (RNA)**, all of the cells of every living thing on Earth contain **DNA** (**deoxyribonucleic acid**). DNA is a complex and long molecule composed of a sequence of smaller molecules, called nucleotides, linked together. Nucleotides are nitrogen-containing molecules, called bases, that are combined with sugar and phosphate. There are four different kinds of nucleotides in DNA. Each nucleotide has a unique base component. The sequence of nucleotides, and therefore of bases, within an organism's DNA is unique. In other words, no two organisms have exactly the same sequence of nucleotides in their DNA, even if they belong to the same species or are related. DNA holds within its nucleotide sequence information that directs the activities of the cell. Groups, or sets of nucleotide sequences that instruct a single function are called genes.

Much of the genetic material, or DNA, of organisms is coiled into compact forms called **chromosomes**. Chromosomes are highly organized compilations of DNA and protein that make the long molecules of DNA more manageable during cell division. In many organisms, including human beings, chromosomes are found within the **nucleus** of a cell. The nucleus is the central compartment of the cell that houses genetic information and acts as a control center for the cell. In other organisms, such as bacteria, DNA is not found within a nucleus. Instead, the DNA (usually in the form of a circular chromosome) chromosome is free within the cell. Additionally, many cells have extrachromosomal DNA that is not found within chromosomes. The mitochondria of cells, and the chloroplasts of plant cells have extrachromosomal DNA that help direct the activities of these organelles independent from the activities of the nucleus where the chromosomes are found. **Plasmids** are circular pieces of extrachromosomal DNA found in bacteria that are extensively used in transgenics.

DNA, whether in chromosomes or in extrachromosomal molecules, uses the same code to direct cell activities. The **genetic code** is the sequence of nucleotides in genes that is defined by sets of three nucleotides. The genetic code itself is universal, meaning it is interpreted the same way in all living things. Therefore, all cells use the same code to store information in DNA, but have different amounts and kinds of information. The entire set of DNA found within a cell (and all of the identical cells of a multicellular organism) is called the genome of that cell or organism.

The DNA of chromosomes within the cellular genome is responsible for the production of proteins. The universal genetic code simply tells cells which proteins to make. Proteins, in turn have many varied and important functions, and in fact help determine the major characteristics of cells and whole organisms. As **enzymes**, proteins carry out thousands of kinds of chemical reactions that make life possible. Proteins also act as cell receptors and signal molecules, which enable cells to communicate with one another, to coordinate growth and other activities important for wound healing and development. Thus, many of the vital activities and characteristics that define a cell are really the result of the proteins that are present. The proteins, in turn, are determined by the genome of the organism.

Because the genetic code with genes is the same for all known organisms, and because genes determine characteristics of organisms, the characteristics of one kind of organism can be transferred to another. If genes from an insect, for example, are placed into a plant in such a way that they are functional, the plant will gain characteristics of the insect. The insect's DNA provides information on how to make insect

proteins within the plant because the genetic code is interpreted in the same way. That is, the insect genes give new characteristics to the plant. This very process has already been performed with firefly genes and tobacco plants. Firefly genes were spliced into tobacco plants, which created new tobacco plants that could glow in the dark. This amazing artificial genetic mixing, called recombinant **biotechnology**, is the crux of transgenics. The organisms that are created from mixing genes from different sources are transgenic. The glow-in-the-dark tobacco plants in the previous example, then, are transgenic tobacco plants.

One of the major obstacles in the creation of transgenic organisms is the problem of physically transferring DNA from one organism or cell into another. It was observed early on that bacteria resistant to **antibiotics** transferred the resistance characteristic to other nearby bacterial cells that were not previously resistant. It was eventually discovered that the resistant bacterial cells were actually exchanging plasmid DNA carrying resistance genes. The **plasmids** traveled between resistant and susceptible cells. In this way, susceptible bacterial cells were transformed into resistant cells.

The permanent modification of a genome by the external application of DNA from a cell of different **genotype** is called **transformation**. Transformed cells can pass on the new characteristics to new cells when they reproduce because copies of the foreign transgenes are replicated during cell division. Transformation can be either naturally occurring or the result of transgenics. Scientists mimic the natural uptake of plasmids by bacterial cells for use in creating transgenic cells. Certain chemicals make transgenic cells more willing to take-up genetically engineered plasmids. Electroporation is a process where cells are induced by an electric current to take up pieces of foreign DNA. Transgenes are also introduced via engineered **viruses**. In a procedure called transfection, viruses that infect bacterial cells are used to inject the foreign pieces of DNA. DNA can also be transferred using microinjection, which uses microscopic needles to insert DNA to the inside of cells. A new technique to introduce transgenes into cells uses liposomes. Liposomes are microscopic spheres filled with DNA that fuse to cells. When liposomes merge with host cells, they deliver the transgenes to the new cell. Liposomes are composed of lipids very similar to the lipids that make up cell membranes, which gives them the ability to fuse with cells.

With the aid of new scientific knowledge, scientists can now use transgenics to accomplish the same results as selective breeding.

By recombining genes, bacteria that metabolize petroleum products are created to clean-up the environment, antibiotics are made by transgenic bacteria on mass industrial scales, and new protein drugs are produced. By creating transgenic plants, food crops have enhanced productivity. Transgenic corn, wheat, and soy with herbicide resistance, for example, are able to grow in areas treated with herbicide that kills weeds. Transgenic tomato plants produce larger, more colorful tomatoes in greater abundance. Transgenics is also used to create **influenza** immunizations and other vaccines.

Despite their incredible utility, there are concerns regarding trangenics. The Human Genome Project is a large collaborative effort among scientists worldwide that announced the determination of the sequence of the entire human genome in 2000. In doing this, the creation of transgenic humans could become more of a reality, which could lead to serious ramifications. Also, transgenic plants used as genetically modified food is a topic of debate. For a variety of reasons, not all scientifically based, some people argue that transgenic food is a consumer safety issue because not all of the effects of transgenic foods have been fully explored.

See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Chromosomes, eukaryotic; Chromosomes, prokaryotic; DNA (Deoxyribonucleic acid); DNA hybridization; Molecular biology and molecular genetics

TRANSLATION

Translation is the process in which genetic information, carried by messenger **RNA** (mRNA), directs the synthesis of proteins from amino acids, whereby the primary structure of the protein is determined by the nucleotide sequence in the mRNA. Although there are some important differences between translation in **bacteria** and translation in eukaryotic cells the overall process is similar. Essentially, the same type of translational control mechanisms that exist in eukaryotic cells do not exist in bacteria.

A molecule known as the ribosome is the site of the **protein synthesis**. The ribosome is protein bound to a second species of RNA known as ribosomal RNA (rRNA). Several **ribosomes** may attach to a single mRNA molecule, so that many polypeptide chains are synthesized from the same mRNA. The ribosome binds to a very specific region of the mRNA called the promoter region. The promoter is upstream of the sequence that will be translated into protein.

The nucleotide sequence on the mRNA is translated into the amino acid sequence of a protein by adaptor molecules composed of a third type of RNA known as transfer RNAs (tRNAs). There are many different species of tRNAs, with each species binding a particular type of amino acid. In protein synthesis, the nucleotide sequence on the mRNA does not specify an amino acid directly, rather, it specifies a particular species of tRNA. Complementary tRNAs match up on the strand of mRNA every three bases and add an amino acid onto the lengthening protein chain. The three base sequence on the mRNA are known as "codons," while the complementary sequence on the tRNA are the "anti-codons."

The ribosomal RNA has two subunits, a large subunit and a small subunit. When the small subunit encounters the mRNA, the process of translation to protein begins. There are two sites in the large subunit, an "A" site, and a "P" site. The start signal for translation is the codon ATG that codes for methionine. A tRNA charged with methionine binds to the translation start signal. After the first tRNA bearing the amino acid appears in the "A" site, the ribosome shifts so that the tRNA is now in the "P" site. A new tRNA molecule corresponding to the codon of the mRNA enters the "A" site. A pep-

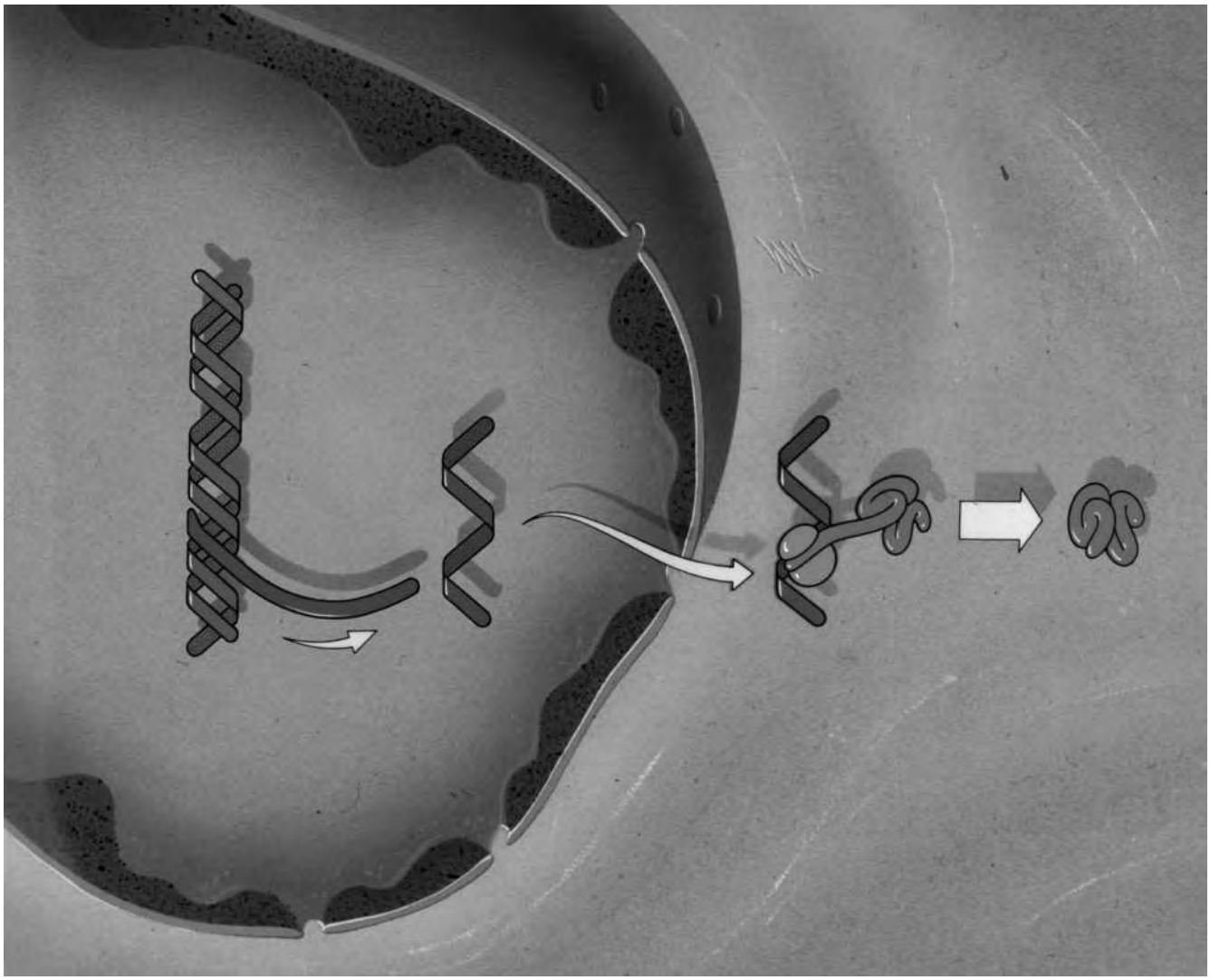


Illustration depicting the transcription of DNA inside the eukaryotic nucleus and the translation of the messenger RNA to form protein that occurs outside the nucleus.

tide bond is formed between the amino acid brought in by the second tRNA and the amino acid carried by the first tRNA. The first tRNA is now released and the ribosome again shifts. The second tRNA bearing two amino acids is now in the "P" site, and a third tRNA can now bind to the "A" site. The process of the tRNA binding to the mRNA aligns the amino acids in a specific order. This long chain of amino acids constitutes a protein. Therefore, the sequence of nucleotides on the mRNA molecule directs the order of the amino acids in a given protein. The process of adding amino acids to the growing chain occurs along the length of the mRNA until the ribosome comes to a sequence of bases that is known as a "stop codon." When that happens, no tRNA binds to the empty "A" site. This is the signal for the ribosome to release the polypeptide chain and the mRNA.

Bacterial ribosomes are smaller than eukaryotic ribosomes. In some cases, bacterial ribosomes contain less than have the total protein found in eukaryotic ribosomes. Bacteria also respond to fewer initiation factors than do eukaryotic cells.

After being released from the tRNA, some proteins may undergo post-translational modifications. They may be cleaved by a proteolytic (protein cutting) enzyme at a specific site. Alternatively, they may have some of their amino acids biochemically modified. After such modifications, the polypeptide forms into its native shape and starts acting as a functional protein in the cell.

There are four different nucleotides, A, U, G and T. If they are taken three at a time (to specify a codon, and thus, indirectly specify an amino acid), 64 codons could be specified. However, there are only 20 different amino acids. Therefore, several triplets code for the same amino acid; for example UAU and UAC both code for the amino acid tyrosine. In addition, some codons do not code for amino acids, but code for polypeptide chain initiation and termination. The

genetic code is non-overlapping, i.e., the nucleotide in one codon is never part of the adjacent codon. The code also seems to be universal in all living organisms.

See also Cell cycle (prokaryotic), genetic regulation of; Chromosomes, prokaryotic; Cytoplasm, prokaryotic; Genetic regulation of prokaryotic cells; Molecular biology and molecular genetics; Protein synthesis; Proteins and enzymes; Ribonucleic acid (RNA)

TRANSMISSION ELECTRON MICROSCOPE (TEM)

• *see* ELECTRON MICROSCOPE, TRANSMISSION AND SCANNING

TRANSMISSION OF PATHOGENS

Microorganisms that cause disease in humans and other species are known as pathogens. The transmission of pathogens to a human or other host can occur in a number of ways, depending upon the microorganism.

A common route is via water. The ingestion of contaminated water introduces the microbes into the digestive system. Intestinal upsets can result. As well, an organism may be capable of entering the cells that line the digestive tract and gaining entry to the bloodstream. From there, an infection can become widely dispersed. A prominent example of a water borne pathogen is *Vibrio cholerae*, the bacterium that causes cholera. The **contamination** of drinking water by this bacterium is still at epidemic proportions in some areas of the world.

Pathogens can also be transmitted via the air. **Viruses** and bacterial spores are light enough to be lifted on the breeze. These agents can subsequently be inhaled, where they cause lung infections. An example of such as virus is the Hanta virus. A particularly prominent bacterial example is the spore form of the anthrax-causing bacterium *Bacillus anthracis*. The latter has also been identified as a bioterrorist weapon that can, as exemplified in a 2001 terrorist attack on the United States, be transmitted in mail that when opened or touched can result in cutaneous or inhalation **anthrax**.

Still other microbial pathogens are transmitted from one human to another via body fluids such as the blood. This route is utilized by a number of viruses. The most publicized example is the transmission of **Human Immunodeficiency Virus** (**HIV**). HIV is generally regarded to be the cause of acquired **immunodeficiency** syndrome. As well, viruses that cause hemorrhagic fever (e.g., Ebola) are transmitted in the blood. If precautions are not taken when handling patients, the caregiver can become infected.

Transmission of pathogens can occur directly, as in the above mechanisms. As well, transmission can be indirect. An intermediate host that harbors the microorganism can transfer the microbes to humans via a bite or by other contact. *Coxiella burnetti*, the bacterium that cause Q-fever, is transmitted to humans from the handling of animals such as sheep. As another example, the trypanosome parasite that causes **sleeping sickness** enters the bloodstream upon the bite of a female mosquito that acts as a vector for the transmission of the parasite.

Transmission of pathogens, such as those affecting poultry, can be promoted by crowded living conditions.

Finally, some viruses are able to transmit infection over long periods of time by become latent in the host. More specifically, the genetic material of viruses such as the hepatitis viruses and the herpes virus can integrate and be carried for decades in the host genome before the symptoms of infections appear.

See also Anthrax, terrorist use as a biological weapon; Bacteria and bacterial infection; Epidemics and pandemics; Yeast, infectious; Zoonoses

TRANSPLANTATION GENETICS AND IMMUNOLOGY

There are several different types of transplantation. An autograft is a graft from one part of the body to another site on the same individual. An isograft is one between individuals that

are genetically alike, as in identical twins. An allograft is a graft between members of the same species but who are not genetically alike. A xenograft is one between members of different species. The allograft we are most familiar with is that of a blood transfusion. Nonetheless, the replacement of diseased organs by transplantation of healthy tissues has frustrated medical science because the **immune system** of the recipient recognizes that the donor organ is not "self" and rejects the new organ.

The ability to discriminate between self and nonself is vital to the functioning of the immune system so it can protect the body from disease and invading **microorganisms**. However, the same immune response that serves well against foreign proteins prevents the use of organs needed for life saving operations. Virtually every cell in the body carries distinctive proteins found on the outside of the cell that identify it as self. Central to this ability is a group of genes that are called the (**MHC**), or **major histocompatibility complex**. The genes that code for those proteins in humans are called the **HLA** or **Human Leukocyte Antigen**. These are broken down to class I (HLA-A, B, and Cw), class II (HLA-DR, DQ, and DP) and class III (no HLA genes).

The MHC was discovered during tumor transplantation studies in mice by Peter Gorer in 1937 at the Lister Institute, and was so named because "histo" stands for tissue in medical terminology. The genes that compose the MHC are unique in that they rarely undergo **recombination** and so are inherited as a haplotype, one from each parent. They are also highly polymorphic. This means that the genes and the molecules they code for vary widely in their structure from one individual to another and so transplants are likely to be identified as foreign and rejected by the body. Scientists have also noted that this area of the genome undergoes more mutational events then other regions, which probably accounts for some of its high degree of polymorphism. As previously mentioned, there are several classes of the MHC. The role of the MHC Class I is to make those proteins that identify the cells of the body as "self," and they are found on nearly every cell in the body that has **nucleus**. Nonself proteins are called antigens and the body first learns to identify self from nonself just before birth, in a **selection** process that weeds out all the immature T-cells that lack self-tolerance. Normally, this process continues throughout the lifespan of the organism. A breakdown in this process leads to **allergies** and at the extreme, results in such autoimmune diseases as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. The job of the Class I proteins is to alert killer **T cells** that certain cells in the body have somehow been transformed, either by a viral infection or cancer, and they need to be eliminated. Killer T-cells will only attack cells that have the same Class I glycoproteins that they carry themselves. The Class II MHC molecules are found on another immunocompetant cell called the B-cells. These cells mature into the cells that make antibodies against foreign proteins. The class II molecules are also found on macrophages and other cells that present foreign antigens to T-helper cells. The Class II antigens combine with the foreign **antigen** and form a complex with the **antibody**, which is subsequently recognized and then eliminated by the body.

The ability of killer T-cells to respond only to those transformed cells that carry Class I antigen, and the ability of helper T-cells to respond to foreign antigens that carry Class II antigen, is called MHC restriction. This is what is tested for when tissues are typed for transplantation. Most transplantation occurs with allogeneic organs, which by definition are those that do not share the same MHC locus. The most sensitive type of transplantation with respect to this are those involving the bone marrow (Haematopoietic Stem Cell Transplantation) HLA matching is an absolute requirement so its use is limited to HLA-matched donors, usually a brother or sister. The major complications include graft-versus-host disease (GvHD is an attack of immunocompetant donor cells to immunosuppressed recipient cells) and rejection, which is the reverse of GvHD. The least sensitive are corneal lens transplantation, probably because of lack of vascularisation in the cornea and its relative immunological privilege. Drugs like cyclosporin A have made transplant surgery much easier, although the long term consequences of suppressing immune function are not yet clear. This antirejection drug is widely used in transplant surgery and to prevent and treat rejection and graft-versus-host disease in bone marrow transplant patients by suppressing their normal immune system. Newer strategies, including **gene** therapy, are being developed to prevent the acute and chronic rejection of transplanted tissues by introducing new genes that are important in preventing rejection. One promising aspect is the delivery of genes that encode foreign donor antigens (alloantigens). This might be an effective means of inducing immunological tolerance in the recipient and eliminate the need for whole-body immunosuppression.

See also Antibody and antigen; Immunogenetics; Immunologic therapies; Immunosuppressant drugs; Major histocompatibility complex (MHC)

TRANSPOSITION

A transposition is a physical movement of genetic material (i.e., **DNA**) within a genome or the movement of DNA across genomes (i.e., from one genome to another). Because these segments of genetic material contain genes, transpositions resulting in changes of the loci (location) or arrangements of genes are **mutations**. Transposition mutations occur in a wide range of organisms. **Transposons** occur in **bacteria**, and transposable elements have been demonstrated to operate in higher eukaryotic organisms, including mammalian systems.

Transposition mutations may only occur if the DNA being moved, termed the transposon, contains intact inverted repeats at its ends (terminus). In addition, functional tranposase **enzymes** must be present.

There are two types or mechanisms of transposition. Replicative transpositions involve the copying of the segment of section DNA to be moved (transposable element) before the segment is actually moved. Accordingly, with replicative transposition, the original section of DNA remains at its original location and only the copy is moved and inserted into its new position. In contrast, with conservative transpositions, the

segment of DNA to be moved is physically cut from its original location and then inserted into a new location. The DNA from which the tranposon is removed is termed the donor DNA, and the DNA to which the transposon is added is termed the receptor DNA.

Transposons are not passive participants in transposition. Transposons carry the genes that code for the enzymes needed for transposition. In essence, they carry the mechanisms of transposition with them as they move or jump (hence Barbara McClintok's original designation of "jumping genes") throughout or across genomes. Transposons carry special **insertion sequences** (IS elements) that carry the genetic information to code for the enzyme transposase that is required to accomplish transposition mutations. One of the most important mechanisms of transposase is that they are the enzymes responsible for cutting the receptor DNA to allow the insertion of the transposon.

Transitions are a radical mutational mechanism. The physical removal of both DNA and genes can severely damage or impair the function of genes located in the transposons (especially those near either terminus). Correspondingly, the donor molecules suffer a deletion of material that may also render the remaining genes inoperative or highly impaired with regard to function.

McClintok's discovery of transposons, also termed "jumping genes" in the late 1940's (before the formation of the Waston-Crick model of DNA) resulted in her subsequent award of a Nobel Prize for Medicine or Physiology.

Transposition segments termed retrotransposons may also utilize an **RNA** intermediate complimentary copy to accomplish their transposition.

Transposition can radically and seriously affect phenotypic characteristics including transfer of **antibiotic resistance** in bacterium. Following insertion, transposed genetic elements usually generate multiple copies of the genes transferred, further increasing their disruption to both the **genotype** and phenotypic expression.

See also Antibiotics; Microbial genetics

TREPONEMA • *see* SYPHILIS

TRYPANOSOME • *see* CHAGAS' DISEASE

TUBERCULOSIS

Tuberculosis (TB) is an infectious disease of the lungs caused by the bacterium *Mycobacterium tuberculosis.* In the mid-nineteenth century, about one-fourth of the mortality rate was attributable to tuberculosis. It was particularly rampant in early childhood and young adulthood. Its presence was felt throughout the world, but by the 1940s, with the introduction of **antibiotics**, there was a sharp decline of cases in developed countries. For less-developed countries with poor **public health** structures, tuberculosis is still a major problem. Since 1989, however, there has been an increase in reported cases in economically advanced countries due mainly to immunosuppression associated with **AIDS**, and the emergence of antibiotic-resistant strains of TB.

The bacillus infects the lungs of those who inhale the infected droplets formed during coughing by an individual who has an active case of the disease. It can also be transmitted by unpasteurized milk, as animals can be infected with the **bacteria**. The disease is dormant in different parts of the body until it becomes active and attacks the lungs, leading to a chronic infection. Symptoms include fatigue, loss of weight, night fevers and chills, and persistent coughing with sputum-streaked blood. The virulent form of the infection can then spread to other parts of the body. Without treatment, the condition is eventually fatal.

Chest x rays and sputum examinations can show the presence of tuberculosis. Tuberculin, a purified protein taken from the tuberculosis bacilli, is placed under the skin of the forearm during a tuberculosis skin test. In two or three days if there is a red swelling at the site, the test is positive, and indicates TB infection, but not necessarily active TB disease. Early detection of the disease facilitates effective treatment to avoid the possibility of it becoming active later on.

Populations at risk of contracting TB are people with certain medical conditions or those using drugs for medical conditions that weaken the **immune system**. Others at risk are low-income groups, people from undeveloped countries with high TB rates, people who work in or are residents of long-term care facilities (nursing homes, prisons, hospitals), those who are significantly underweight, alcoholics, and intravenous drug users.

Traces of lesions from tuberculosis have been found in the lungs of ancient Egyptian mummies. The recent discovery of a Pre-Columbian mummy has resolved the debate on whether or not European explorers introduced the disease to the New World. Lung samples from a Peruvian woman who lived 500 years before Columbus discovered America show a lump that was identified as tuberculosis by **DNA** testing. Hippocrates, a Greek physician who lived from 460 to 370 B.C., described the disease. The Greek name for the disease was *phthisis,* derived from the verb *phthinein,* meaning to waste away. Tuberculosis was also called consumption because of the wasting away effects (notably, significant losses of weight over a period of time) of the disease.

In 1839, Johann Schonlein is credited with first labeling the disease tuberculosis. In 1882, the tubercle bacillus was discovered by **Robert Koch**, the German physician who pioneered the science of bacteriology. This landmark discovery was followed eight years later by his extraction of a protein from dead bacilli called tuberculin. This protein is still used to test for the presence of TB infection in a dormant or early stage. Another important diagnostic breakthrough came in 1895 with the discovery of Wilhelm Conrad Roentgen's x rays. The presence of TB lesions was detected on x rays.

Two twentieth century French scientists, Albert Calmette and Camille Guerin, developed a **vaccine** against tuberculosis from a weakened strain of bovine bacillus. Called BCG for Bacillus-Calmette-Guerin, this vaccine is the only

Hospital for tuberculosis patients in Turkey.

one still in use although some scientists question its effectiveness. Despite doubts about the vaccine, it is still widely used, especially in TB endemic countries where other preventive measures are lacking. The U.S. Public Health Service's policy recommends testing and drug therapy for those infected instead of **vaccination**. The two factors responsible for this policy are the low incidence of TB in the United States and the doubts raised about BCG. The **Centers for Disease Control** and Prevention, (CDC), however, in its concern over the rising incidence of TB in the United States and the appearance of multidrug-resistant tuberculosis (MDR TB) which is difficult to treat, reexamined the TB vaccination issue, and released recommendations for its use in limited situations.

The CDC still recommends the use of skin tests and drug therapy as the most important measures in controlling the incidence of TB in the United States. Drug therapy is 90% effective in halting the infection. Since those vaccinated test positive with the skin test, a vaccination program would interfere with skin testing. Mass vaccination would risk giving up a simple test that provides an early warning. Relying on the drug treatment program to stop TB **epidemics**, however, has one major drawback. The drug therapy takes six months to a year before halting the infection. People infected are often among the homeless, poor, drug addicted, or criminal societies. Unless these people are carefully supervised to make sure they complete a regimen of drug therapy, it is difficult to effect a cure for the disease.

Throughout the nineteenth century and up until the 1960s, physicians sent their TB patients to sanatoriums which were rest homes located in mountains or semi-arid regions such as the American southwest. These locations were supposed to help the breathing process by providing clean and dry air. Physicians assumed that deeper, easier breathing in a work-free environment would help overcome the disease. Prior to the advent of antibiotics, these retreats were the only recourse for chronically ill tubercular patients. Although treatment in sanatoriums did help many, they were phased out before the 1960s, and replaced by antibiotic drug **chemother-**

apy, which could be administered in either a hospital or home environment. Over 90% of TB patients can be cured by a combination of inexpensive antibiotics, but it is necessary they be used for a period of at least six months.

The impact of tuberculosis was evident in the nineteenth and early twentieth centuries in literature, art, and music. Puccini's opera, *La Boheme,* was created around the tragic death of the tubercular heroine, Mimi. Since TB often attacked the young, many poets, artists and musicians fell prey to the disease before they had a chance to fulfill their creative work. Among them, Amedeo Modigliani, John Keats, Frederic Chopin, and Anton Chekhov were claimed by the disease, along with millions of other young people during the period. In the United States, American playwright Eugene O'Neill was one of the fortunate few who did recover in a sanatorium and went on to write his plays. His early play, *The Straw,* written in 1919, dramatically shows what life was like in a sanatorium.

In the past, U.S. city and state governments were actively involved in regulations that controlled infected people from spreading the infection. At present, federal, state, and local agencies must again take a leading role in formulating a public policy on this complicated health problem. Several states are using a program called Directly Observed Therapy (DOT) to combat the rising incidence of TB. This program has met with considerable success in lowering reported cases of TB as much as 15% in New York City during the late 1990s.

DOT is offered at soup kitchens, clinics, hospitals, neighborhood health centers, and drug rehabilitation centers. Outreach workers enable those with TB to get help with the least amount of red tape. The wide array of medicines needed to treat the disease are made available, and ample funding has been provided from federal, state, and local agencies. Apartments are located for homeless patients and special provisions are made to help released prison inmates and those on parole. Guidelines for compassionate, supervised medical services are periodically reviewed for the successful implementation of the DOT program.

Despite such measures, the U.S. Department of Health and Human Services predicts tuberculosis, will spread further by the year 2005. In 1990, there were 7,537,000 TB cases worldwide. That number is expected to rise to 11,875,000 in 2005, a 58% increase. Most of the rise in rate is attributed to demographic factors (77%) while 23% accounts for the epidemiological factors, i.e., the rise in **HIV** infection. Approximately 30 million people around the world will die of TB from 2000 to 2009. These predictions are considered conservative because many cases of TB are never reported.

See also AIDS, recent advances in research and treatment; Bacteria and bacterial infection; Epidemiology, tracking diseases with technology; Public health, current issues

TULAREMIA

Tularemia is a plague-like disease caused by the bacterium *Francisella tularensis* that can transferred to man from animals such as rodents, voles, mice, squirrels, and rabbits. Reflecting the natural origin of the disease, tularemia is also known as rabbit fever. Indeed, the rabbit is the most common source of the disease. Transfer of the bacterium via contaminated water and vegetation is possible as well.

The disease can easily spread from the environmental source to humans (although direct person-to-person contact has not been documented). This contagiousness and the high death rate among those who contract the disease made the bacterium an attractive bioweapon. Both the Japanese and Western armies experimented with *Francisella tularensis* during World War II. Experiments during and after that war established the devastating effect that aerial dispersion of the **bacteria** could exact on a population. Until the demise of the Soviet Union, its biological weapons development program actively developed strains of the bacterium that were resistant to **antibiotics** and vaccines.

Tularemia naturally occurs over much of North America and Europe. In the United States, the disease is predominant in south-central and western states such as Missouri, Arkansas, Oklahoma, South Dakota, and Montana. The disease almost always occurs in rural regions. The animal reservoirs of the bacterium become infected typically by a bite from a blood-feeding tick, fly, or mosquito.

The causative bacterium, *Francisella tularensis,* is a Gram-negative bacterium that, even though it does not form a spore, can survive for protracted periods of time in environments such as cold water, moist hay, soil, and decomposing carcasses.

The number of cases of tularemia in the world is not known, as accurate statistics have not been kept, and because illnesses attributable to the bacterium go unreported. In the United States, the number of cases used to be high. In the 1950s thousands of people were infected each year. This number has dropped considerable, to less than 200 each year in the 1990s and those who are infected now tend to be those who are exposed to the organism in its rural habitat (e.g., hunters, trappers, farmers, and butchers).

Humans can acquire the infection through breaks in the skin and mucous membranes, by ingesting contaminated water, or by inhaling the organism. An obligatory step in the establishment of an infection is the invasion of host cells. A prime target of invasion is the immune cell known as macrophages. Infections can initially become established in the lymph nodes, lungs, spleen, liver, and kidney. As these infections become more established, the microbe can spread to tissues throughout the body.

Symptoms of tularemia vary depending on the route of entry. Handling an infected animal or carcass can produce a slow-growing ulcer at the point of initial contact and swollen lymph nodes. When inhaled, the symptoms include the sudden development of a headache with accompanying high fever, chills, body aches (particularly in the lower back), and fatigue. Ingestion of the organism produces a sore throat, abdominal pain diarrhea, and vomiting. Other symptoms can include eye infection and the formation of skin ulcers. Some people also develop **pneumonia**-like chest pain. An especially severe pneumonia develops from the inhalation of one type of the organism, which is designated as *Francisella tularensis biovar*

tularensis (type A). The pneumonia can progress to respiratory failure and death. The symptoms typically tend to appear three to five days after entry of the microbe into the body.

The infection responds to antibiotic treatment and recovery can be complete within a few weeks. Recovery produces a long-term **immunity** to re-infection. Some people experience a lingering impairment in the ability to perform physical tasks. If left untreated, tularemia can persist for weeks, even months, and can be fatal. The severe form of tularemia can kill up to 60% of those who are infected if treatment is not given.

A **vaccine** is available for tularemia. To date this vaccine has been administered only to those who are routinely exposed to the bacterium (e.g., researchers). The potential risks of the vaccine, which is a weakened form of the bacterium, have been viewed as being greater than the risk of acquiring the infection.

See also Bacteria and bacterial infection; Bioterrorism, protective measures; Infection control; Zoonoses

TUMOR VIRUSES

Tumor **viruses** are those viruses that are able to infect cells and cause changes within the cell's operating machinery such that the cell's ability to regulate its growth and division is destroyed and the cells become cancerous.

Human papillomavirus, **hepatitis** B, **Epstein-Barr virus**, **human T-cell leukemia virus**, SV-40, and Rous sarcoma virus are all tumor viruses.

The ability of the Rous sarcoma virus to cause sarcomas (cancers of connective tissue) has been known since 1911, when **Peyton Rous** demonstrated that a sarcoma material from chicken could be filtered and the filtered fluid was still capable of inducing the cancer. The virus was both the first oncogenic (cancer-causing) virus to be discovered and (although not known until much later) the first retrovirus to be discovered. Another, well-known example of a retrovirus is **HIV**.

There are some 90 types of human papillomavirus, based upon the genetic sequence of their genomes. The target of the viral infection is a certain type of epithelial cell known as stratified squamous epithelium. The cells can be located on the surface of the skin, or can be mucosal cells in regions of the body such as the genital tract. For example, two human papillomaviruses are the most common cause of genital warts. While these warts are noncancerous, other types of papillomavirus result in the development of cervical cancer. Furthermore, human papillomavirus types 16 and 18 are the main cause of genital tract malignancies. The virus is transmitted from person to person typically via sexual contact.

How human papillomavirus triggers the uncontrolled growth that is a hallmark of cancerous cells is still unknown. Studies have determined that in cells that have not yet become cancerous, the viral genetic material is not associated with the cell's genetic material, and that production of new virus particles is still occurring. However, in cancerous cells the viral genetic material has been integrated into that of the host and no new virus particles are being made. Whether the integration event is a trigger for cancerous growth is not known.

The hepatitis B virus is associated with liver damage and liver cancer. The virus is transmitted from person to person via contaminated blood (which commonly occurs via sharing of needles), breast milk, and possibly saliva. Over 90% of all hepatitis B infections are cleared as the **immune system** responds to the infection. However, in some 5% of those infected the infection becomes chronic. Infected individuals can be asymptomatic, but remain carriers of the virus and thus able to pass on the virus to others.

Chronic infection with the hepatitis B virus greatly elevates the chances of developing cancer of the liver. Because the virus can be present for decades before the damage of liver cancer is diagnosed, the best strategy is the preemptive use of hepatitis B **vaccine** in those offspring born to mothers who are known to be positive for the virus.

As for human papillomavirus, the molecular mechanism by which hepatitis B virus triggers cancerous growth of cells is unknown. The periodic response of the immune system to the virus may over time favor the expression of genes whose products are involved in overriding growth and division control mechanisms.

The Epstein-Barr virus is linked to two specific cancers. One is called Burkitt's lymphoma, a cancer of the B-cell components of the immune system. The lymphoma is a common cancer of children and occurs almost exclusively in the central region of Africa. The region's high rate of **malaria** may play a role in the prevalence of the lymphoma, as malaria causes an increase in the number of the already-infected B-cells. The rapid increase in the virally infected B-cells might cause a genetic malfunction that leads to tumor development. The second cancer associated with the virus is nasopharyngeal carcinoma. This cancer is restricted to the coastal region of China, for as yet unknown reasons.

Human T-cell leukemia virus causes cancer in the T-cell components of the immune system. Infection is widespread in Japan and areas of Africa, and is spreading to western nations including the United States.

A virus designated SV-40, which is harbored by species of monkeys, is isolated from a sizable number of cancer sufferers.

See also Oncogene; Oncogenetic research

TYPHOID FEVER

Typhoid fever is a severe infection causing a sustained high fever, and caused by the **bacteria** *Salmonella typhi*—similar to the bacteria spread by chicken and eggs resulting in "Salmonella poisoning," or food poisoning. *S. typhi* bacteria, however, do not multiply directly in food, as do the **Salmonella** responsible for food poisoning, nor does it have vomiting and diarrhea as the most prominent symptoms. Instead, persistently high fever is the hallmark of infection with *Salmonella typhi*.

Mary Mallon ("Typhoid Mary") spread the typhoid bacterium before she was quarantined.

S. typhi bacteria are passed into the stool and urine of infected patients, and may continue to be present in the stool of asymptomatic carriers (individuals who have recovered from the symptoms of the disease, but continue to carry the bacteria). This carrier state occurs in about 3% of all individuals recovered from typhoid fever.

The disease is passed between humans, then, through poor **hygiene**, such as deficient hand washing after toileting. Individuals who are carriers of the disease and who handle food can be the source of epidemic spread of typhoid. One such individual was the inspiration for the expression "Typhoid Mary," a name given to someone with whom others wish to avoid all contact. The real "Typhoid Mary" was a cook named Mary Mallon (1855–1938) who lived in New York City around 1900. She was a carrier of typhoid and was the cause of at least 53 outbreaks of typhoid fever.

Typhoid fever is a particularly difficult problem in parts of the world with less-than-adequate sanitation practices. In the United States, many patients who become afflicted with typhoid fever have recently returned from travel to another country, where typhoid is much more prevalent, such as Mexico, Peru, Chile, India, and Pakistan.

To cause disease, the *S. typhi* bacteria must be ingested. This often occurs when a carrier does not wash hands sufficiently well after defecation, and then serves food to others. In countries where open sewage is accessible to flies, the insects land on the sewage, pick up the bacteria, and then land on food to be eaten by humans.

Ingested bacteria travel down the gastrointestinal tract, where they are taken in by cells called mononuclear phagocytes. These phagocytes usually serve to engulf and kill invading bacteria and **viruses**. However, in the case of *S. typhi*, the bacteria survive ingestion by the phagocytes, and multiply within these cells. This period of time, during which the bacteria are multiplying within the phagocytes, is the 10–14 day incubation period. When huge numbers of bacteria fill an individual **phagocyte**, the bacteria are discharged out of the cell and into the bloodstream, where their presence begins to cause symptoms.

The presence of increasingly large numbers of bacteria in the bloodstream (called bacteremia) is responsible for an increasingly high fever, which lasts throughout the four to eight weeks of the disease, in untreated individuals. Other symptoms include constipation (initially), extreme fatigue, headache, a rash across the abdomen known as "rose spots," and joint pain.

The bacteria move from the bloodstream into certain tissues of the body, including the gallbladder and lymph tissue of the intestine (called Peyer's patches). The tissue's inflammatory response to this invasion causes symptoms ranging from **inflammation** of the gallbladder (cholecystitis) to intestinal bleeding and actual perforation of the intestine. Perforation of the intestine refers to an actual hole occurring in the wall of the intestine, with leakage of intestinal contents into the abdominal cavity. This causes severe irritation and inflammation of the lining of the abdominal cavity, called peritonitis, which is frequently a fatal outcome of typhoid fever.

Other complications of typhoid fever include liver and spleen enlargement (sometimes so extreme that the spleen ruptures), anemia (low red blood cell count due to blood loss from the intestinal bleeding), joint infections (especially frequent in patients with sickle cell anemia and **immune system** disorders), **pneumonia** (due to a superimposed infection, usually by Streptococcus pneumoniae), heart infections, **meningitis**, and infections of the brain (causing confusion and even coma). Untreated typhoid fever may take several months to resolve fully.

Samples of a patient's stool, urine, blood, and bone marrow can all be used to **culture** (grow) the *S. typhi* bacteria in a laboratory for identification under a **microscope**. These types of cultures are the most accurate methods of diagnosis.

Chloramphenicol is the most effective drug treatment for *S. typhi*, and symptoms begin to improve slightly after only 24–48 hours of receiving the medication. Another drug, ceftriaxone, has been used recently, and is extremely effective, lowering fever fairly quickly.

Carriers of *S. typhi* must be treated even when asymptomatic, as they are responsible for the majority of new cases of typhoid fever. Eliminating the carrier state is actually a difficult task, and requires treatment with one or even two different medications for four to six weeks. In the case of a carrier with gall stones, surgery may need to be performed to remove the gall bladder, because the *S. typhi* bacteria are often housed in the gall bladder, where they may survive despite antibiotic treatment.

Hygienic sewage disposal systems in a community, as well as hygienic personal practices, are the most important factors in preventing typhoid fever. For travelers who expect to go to countries where *S. typhi* is a known **public health** problem, immunizations are available. Some of these immunizations provide only short-term protection (for a few months), while others may be protective for several years. Immunizations that provide a longer period of protection, with fewer side effects from the **vaccine** itself, are being developed.

See also Antibody formation and kinetics; Bacteria and bacterial infection; Immunity, active, passive and delayed; Immunochemistry; Immunogenetics; Immunology; Vaccination

TYPHUS

Typhus is a disease caused by a group of **bacteria** called **Rickettsia**. Three forms of typhus are recognized: epidemic typhus, a serious disease that is fatal if not treated promptly; rat-flea or endemic typhus, a milder form of the disease; and scrub typhus, another fatal form. The Rickettsia species of bacteria that cause all three forms of typhus are transmitted by insects. The bacteria that cause epidemic typhus, for instance, are transmitted by the human body louse; the bacteria that cause endemic typhus are transmitted by the Oriental rat flea; and bacteria causing scrub typhus are transmitted by chiggers.

Typhus takes its name from the Greek word *typhos* meaning smoke, a description of the mental state of infected persons. Typhus is marked by a severe stupor and delirium, as well as headache, chills, and fever. A rash appears within four to seven days after the onset of the disease. The rash starts on the trunk and spreads to the extremities. In milder forms of typhus, such as endemic typhus, the disease symptoms are not severe. In epidemic and scrub typhus, however, the symptoms are extreme, and death can result from complications such as stroke, renal failure, and circulatory disturbances. Fatality can be avoided in these forms of typhus with the prompt administration of **antibiotics**.

Epidemic typhus is a disease that has played an important role in history. Because typhus is transmitted by the human body louse, **epidemics** of this disease break out when humans are in close contact with each other under conditions in which the same clothing is worn for long periods of time. Cold climates also favor typhus epidemics, as people will be more likely to wear heavy clothing in colder conditions. Typhus seems to be a disease of war, poverty, and famine. In fact, according to one researcher, Napoleon's retreat from Moscow in the early nineteenth century was beset by typhus. During World War I, more than three million Russians died of typhus, and during the Vietnam war, sporadic epidemics killed American soldiers.

Epidemic typhus is caused by *Rickettsia prowazekii*. Humans play a role in the life cycle of the bacteria. Lice become infected with the bacteria by biting an infected human; these infected lice then bite other humans. A distinguishing feature of typhus disease transmission is that the louse bite itself does not transmit the bacteria. The feces of the lice are infected with bacteria; when a person scratches a louse bite, the lice feces that have been deposited on the skin are introduced into the bloodstream.

If not treated promptly, typhus is fatal. Interestingly, a person who has had epidemic typhus can experience a relapse of the disease years after they have been cured of their infection. Called Brill-Zinsser disease, after the researchers who discovered it, the relapse is usually a milder form of typhus, which is treated with antibiotics. However, a person with Brill-Zinsser disease can infect lice, which can in turn infect other humans. Controlling Brill-Zinsser relapses is important in stopping epidemics of typhus before they start, especially in areas where lice infestation is prominent.

Endemic typhus is caused by *R. typhi*. These bacteria are transmitted by the Oriental rat flea, an insect that lives on small rodents. Endemic typhus (sometimes called murine typhus or rat-flea typhus) is found worldwide. The symptoms of endemic typhus are mild compared to those of epidemic typhus. In fact, many people do not seek treatment for their symptoms, as the rash that accompanies the disease may be short-lived. Deaths from endemic typhus have been documented, however; these deaths usually occur in the elderly and in people who are already sick with other diseases.

Scrub typhus is caused by *R. tsutsugamushi*, which is transmitted by chiggers. The term "scrub typhus" comes from the observation that the disease is found in habitats with scrub vegetation, but the name is somewhat of a misnomer. Scrub typhus is found in beach areas, savannas, tropical rains forests, deserts, or anywhere chiggers live. Scientists studying scrub typhus label a habitat that contains all the elements that might prompt an outbreak of the disease a "scrub typhus island." A scrub typhus island contains chiggers, rats, vegetation that will sustain the chiggers, and, of course, a reservoir of *R. tsutsugamushi*. Scrub typhus islands are common in the geographic area that includes Australia, Japan, Korea, India, and Vietnam.

The rash that occurs in scrub typhus sometimes includes a lesion called an eschar. An eschar is a sore that develops around the chigger bite. Scrub typhus symptoms of fever, rash, and chills may evolve into stupor, **pneumonia**, and circulatory failure if antibiotic treatment is not administered. Scrub typhus, like epidemic typhus, is fatal if not treated.

Prevention of typhus outbreaks takes a two-pronged approach. Eliminating the carriers and reservoirs of

Rickettsia is an important step in prevention. Spraying with insecticides, rodent control measures, and treating soil with insect-repellent chemicals have all been used successfully to prevent typhus outbreaks. In scrub typhus islands, cutting down vegetation has been shown to lessen the incidence of scrub typhus. The second preventative prong is protecting the body from insect bites. Wearing heavy clothing when venturing into potentially insect-laden areas is one way to protect against insect bites; applying insect repellent to the skin is another. Proper personal **hygiene**, such as frequent bathing and changing of clothes, will eliminate human body lice and thus prevent epidemic typhus. A typhus **vaccine** is also available; however, this vaccine only lessens the severity and shortens the course of the disease, and does not protect against infection.

See also Bacteria and bacterial infection; Bioterrorism, protective measures; Infection control; Zoonoses

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ULTRA-VIOLET STERILIZATION • *see* STERILIZATION

UREY, HAROLD (1893-1981)

American biochemist

Already a scientist of great honor and achievement, Harold Urey's last great period of research brought together his interests and experiences in a number of fields of research to which he devoted his life. The subject of that research was the **origin of life** on Earth.

Urey hypothesized that the earth's primordial atmosphere consisted of reducing gases such as hydrogen, ammonia, and methane. The energy provided by electrical discharges in the atmosphere, he suggested, was sufficient to initiate chemical reactions among these gases, converting them to the simplest compounds of which living organisms are made, amino acids. In 1953, Urey's graduate student Stanley Lloyd Miller carried out a series of experiments to test this hypothesis. In these experiments, an electrical discharge passed through a glass tube containing only reducing gases resulted in the formation of amino acids.

The **Miller-Urey experiment** is a classic experiment in biology. The experiment established that the conditions that existed in Earth's primitive atmosphere were sufficient to produce amino acids, the subunits of proteins comprising and required by living organisms. In essence, the Miller-Urey experiment fundamentally established that Earth's primitive atmosphere was capable of producing the building blocks of life from inorganic materials.

The Miller-Urey experiment also remains the subject of scientific debate. Scientists continue to explore the nature and composition of Earth's primitive atmosphere and thus, continue to debate the relative closeness of the conditions of the experimental conditions to Earth's primitive atmosphere.

The Miller-Urey experiment was but one part of a distinguished scientific career for Urey. In 1934, Harold Urey was awarded the Nobel Prize in chemistry for his discovery of deuterium, an isotope, or species, of hydrogen in which the atoms weigh twice as much as those in ordinary hydrogen. Also known as heavy hydrogen, deuterium became profoundly important to future studies in many scientific fields, including chemistry, physics, and medicine. Urey continued his research on isotopes over the next three decades, and during World War II his experience with deuterium proved invaluable in efforts to separate isotopes of uranium from each other in the development of the first atomic bombs. Later, Urey's research on isotopes also led to a method for determining the earth's atmospheric temperature at various periods in past history. This experimentation has become especially relevant because of concerns about the possibility of global climate change.

Harold Clayton Urey was born in Walkerton, Indiana. His father, Samuel Clayton Urey, was a schoolteacher and lay minister in the Church of the Brethren. His mother was Cora Reinoehl Urey. After graduating from high school, Urey hoped to attend college but lacked the financial resources to do so. Instead, he accepted teaching jobs in country schools, first in Indiana (1911–1912) and then in Montana (1912–1914) before finally entering Montana State University in September of 1914 at the age of 21. Urey was initially interested in a career in biology, and the first original research he ever conducted involved a study of **microorganisms** in the Missoula River. In 1917, he was awarded his bachelor of science degree in zoology by Montana State.

The year Urey graduated also marked the entry of the United States into World War I. Although he had strong pacifist beliefs as a result of his early religious training, Urey acknowledged his obligation to participate in the nation's war effort. As a result, he accepted a job at the Barrett Chemical Company in Philadelphia and worked to develop high explosives. In his Nobel Prize acceptance speech, Urey said that this experience was instrumental in his move from industrial chemistry to academic life.

At the end of the war, Urey returned to Montana State University where he began teaching chemistry. In 1921 he decided to resume his college education and enrolled in the doctoral program in physical chemistry at the University of California at Berkeley. His faculty advisor at Berkeley was the great physical chemist Gilbert Newton Lewis. Urey received his doctorate in 1923 for research on the calculation of heat capacities and entropies (the degree of randomness in a system) of gases, based on information obtained through the use of a spectroscope. He then left for a year of postdoctoral study at the Institute for Theoretical Physics at the University of Copenhagen where Niels Bohr, a Danish physicist, was researching the structure of the atom. Urey's interest in Bohr's research had been cultivated while studying with Lewis, who had proposed many early theories on the nature of chemical bonding.

Upon his return to the United States in 1925, Urey accepted an appointment as an associate in chemistry at the Johns Hopkins University in Baltimore, a post he held until 1929. He interrupted his work at Johns Hopkins briefly to marry Frieda Daum in Lawrence, Kansas, in 1926. Daum was a bacteriologist and daughter of a prominent Lawrence educator. The Ureys later had four children.

In 1929, Urey left Johns Hopkins to become associate professor of chemistry at Columbia University, and in 1930, he published his first book, *Atoms, Molecules, and Quanta,* written with A. E. Ruark. Writing in the *Dictionary of Scientific Biography,* Joseph N. Tatarewicz called this work "the first comprehensive English language textbook on atomic structure and a major bridge between the new quantum physics and the field of chemistry." At this time he also began his search for an isotope of hydrogen. Since Frederick Soddy, an English chemist, discovered isotopes in 1913, scientists had been looking for isotopes of a number of elements. Urey believed that if an isotope of heavy hydrogen existed, one way to separate it from the ordinary hydrogen isotope would be through the vaporization of liquid hydrogen. Urey's subsequent isolation of deuterium made Urey famous in the scientific world, and only three years later he was awarded the Nobel Prize in chemistry for his discovery.

During the latter part of the 1930s, Urey extended his work on isotopes to other elements besides hydrogen. Urey found that the mass differences in isotopes can result in modest differences in their reaction rates

The practical consequences of this discovery became apparent during World War II. In 1939, word reached the United States about the discovery of nuclear fission by the German scientists Otto Hahn and Fritz Strassmann. The military consequences of the Hahn-Strassmann discovery were apparent to many scientists, including Urey. He was one of the first, therefore, to become involved in the U.S. effort to build a nuclear weapon, recognizing the threat posed by such a weapon in the hands of Nazi Germany. However, Urey was deeply concerned about the potential destructiveness of a fission weapon. Actively involved in political topics during the 1930s, Urey was a member of the Committee to Defend America by Aiding the Allies and worked vigorously against the fascist regimes in Germany, Italy, and Spain. He explained

Harold Urey won the 1934 Nobel Prize in Chemistry for his discovery of heavy hydrogen (deuterium).

the importance of his political activism by saying that "no dictator knows enough to tell scientists what to do. Only in democratic nations can science flourish."

Urey worked on the Manhattan Project to build the nation's first atomic bomb. As a leading expert on the separation of isotopes, Urey made critical contributions to the solution of the Manhattan Project's single most difficult problem, the isolation of ^{235}uranium.

At the conclusion of World War II, Urey left Columbia to join the Enrico Fermi Institute of Nuclear Studies at the University of Chicago where Urey continued to work on new applications of his isotope research. During the late 1940s and early 1950s, he explored the relationship between the isotopes of oxygen and past planetary climates. Since isotopes differ in the rate of chemical reactions, Urey said that the amount of each oxygen isotope in an organism is a result of atmospheric temperatures. During periods when the earth was warmer than normal, organisms would take in more of a lighter isotope of oxygen and less of a heavier isotope. During cool periods, the differences among isotopic concentrations would not be as great. Over a period of time, Urey was able to develop a scale, or an "oxygen thermometer," that related the relative concentrations of oxygen isotopes in the shells of sea animals with atmospheric temperatures. Some of those studies continue to

be highly relevant in current research on the possibilities of global climate change.

In the early 1950s, Urey became interested in yet another subject: the chemistry of the universe and of the formation of the planets, including Earth. One of his first papers on this topic attempted to provide an estimate of the relative abundance of the elements in the universe. Although these estimates have now been improved, they were remarkably close to the values modern chemists now accept.

In 1958, Urey left the University of Chicago to become Professor at Large at the University of California in San Diego at La Jolla. At La Jolla, his interests shifted from original scientific research to national scientific policy. He became extremely involved in the U.S. space program, serving as the first chairman of the Committee on Chemistry of Space and Exploration of the Moon and Planets of the National Academy of Science's Space Sciences Board. Even late in life, Urey continued to receive honors and awards from a grateful nation and admiring colleagues.

See also Cell cycle and cell division; Evolution and evolutionary mechanisms; Evolutionary origin of bacteria and viruses

V

VACCINATION

Vaccination refers to a procedure in which the presence of an **antigen** stimulates the formation of antibodies. The antibodies act to protect the host from future exposure to the antigen. Vaccination is protective against infection without the need of suffering through a bout of a disease. In this artificial process an individual receives the antibody-stimulating compound either by injection or orally.

The technique of vaccination has been practiced since at least the early decades of the eighteenth century. Then, a common practice in Istanbul was to retrieve material from the surface sores of a **smallpox** sufferer and rub the material into a cut on another person. In most cases, the recipient was spared the ravages of smallpox. The technique was refined by **Edward Jenner** into a **vaccine** for **cowpox** in 1796.

Since Jenner's time, vaccines for a variety of bacterial and viral maladies have been developed. The material used for vaccination is one of four types. Some vaccines consist of living but weakened **viruses**. These are called attenuated vaccines. The weakened virus does not cause an infection but does illicit an immune response. An example of a vaccination with attenuated material is the **measles**, **mumps**, and rubella (MMR) vaccine. Secondly, vaccination can involve killed viruses or **bacteria**. The biological material must be killed such that the surface is not altered, in order to preserve the true antigenic nature of the immune response. Also, the vaccination utilizes agents, such as alum, that act to enhance the immune response to the killed target. Current thought is that such agents operate by "presenting" the antigen to the **immune system** in a more constant way. The immune system "sees" the target longer, and so can mount a more concerted response to it. A third type of vaccination involves an inactivated form of a toxin produced by the target bacterium. Examples of such so-called toxoid vaccines are the **diphtheria** and **tetanus** vaccines. Lastly, vaccination can also utilize a synthetic conjugate compound constructed from portions of two antigens. The Hib vaccine is an example of such a biosynthetic vaccine.

During an infant's first two years of life, a series of vaccinations is recommended to develop protection against a number of viral and bacterial diseases. These are **hepatitis** B, polio, measles, mumps, rubella (also called German measles), **pertussis** (also called whooping cough), diphtheriae, tetanus (lockjaw), *Haemophilus influenzae* type b, pneumococcal infections, and chickenpox. Typically, vaccination against a specific microorganism or groups of organisms is repeated three or more times at regularly scheduled intervals. For example, vaccination against diphtheria, tetanus, and pertussis is typically administered at two months of age, four months, six months, 15–18 months, and finally at four to six years of age.

Often, a single vaccination will not suffice to develop **immunity** to a given target antigen. For immunity to develop it usually takes several doses over several months or years. A series of vaccinations triggers a greater production of **antibody** by the immune system, and primes the antibody producing cells such that they retain the memory (a form of protein coding and **antibody formation**) of the stimulating antigen for along time. For some diseases, this memory can last for a lifetime following the vaccination schedule. For other diseases, such as tetanus, adults should be vaccinated every ten years in order to keep their body primed to fight the tetanus microorganism. This periodic vaccination is also referred to as a booster shot. The use of booster vaccinations produces a long lasting immunity.

Vaccination acts on the lymphocyte component of the immune system. Prior to vaccination there are a myriad of lymphocytes. Each one recognizes only a single protein or bit of the protein. No other lymphocyte recognizes the same site. When vaccination occurs, a lymphocyte will be presented with a recognizable protein target. The lymphocyte will be stimulated to divide and some of the daughter cells will begin to produce antibody to the protein target. With time, there will be many daughter lymphocytes and much antibody circulating in the body.

With the passage of more time, the antibody production ceases. But the lymphocytes that have been produced still retain the memory of the target protein. When the target is pre-

Vaccination via injection.

sented again to the lymphocytes, as happens in the second vaccination in a series, the many lymphocytes are stimulated to divide into daughter cells, which in turn form antibodies. Thus, the second time around, a great deal more antibody is produced. The antibody response also becomes highly specific for the target. For example, if the target is a virus that causes polio, then a subsequent entry of the virus into the body will trigger a highly specific and prompt immune response, which is designed to quell the invader.

Most vaccinations involve the injection of the immune stimulant. However, oral vaccination has also proven effective and beneficial. The most obvious example is the oral vaccine to polio devised by **Albert Sabin**. Oral vaccination is often limited by the passage of the vaccine through the highly acidic stomach. In the future it is hoped that the bundling of the vaccine in a protective casing will negate the damage caused by passage trough the stomach. Experiments using bags made out of lipid molecules (liposomes) have demonstrated both protection of the vaccine and the ability to tailor the liposome release of the vaccine.

The nature of vaccination, with the use of living or dead material that stimulates the immune system, holds the potential for side effects. For some vaccines, the side effects are minor. For example, a person may develop a slight ache and redness at the site of injection. In some very rare cases, however, more severe reactions can occur, such as convulsions and high fever. However, while there will always be a risk of an adverse reaction from any vaccination, the risk of developing disease is usually far greater than the probability of experiencing severe side effects.

See also Adjuvant; Anti-adhesion methods; Immune stimulation, as a vaccine

VACCINE

A vaccine is a medical preparation given to provide **immunity** from a disease. Vaccines use a variety of different substances ranging from dead **microorganisms** to genetically engineered antigens to defend the body against potentially harmful microorganisms. Effective vaccines change the **immune system** by promoting the development of antibodies that can quickly and effectively attack a disease causing microorganism when it enters the body, preventing disease development.

The development of vaccines against diseases ranging from polio and **smallpox** to **tetanus** and **measles** is considered among one of the great accomplishments of medical science.

Contemporary researchers are continually attempting to develop new vaccinations against such diseases as Acquired Immune Deficiency Syndrome (**AIDS**), cancer, **influenza**, and other diseases.

Physicians have long observed that individuals who were exposed to an infectious disease and survived were somehow protected against that disease in the future. Prior to the invention of vaccines, however, infectious diseases swept through towns, villages, and cities with a horrifying vengeance.

The first effective vaccine was developed against smallpox, an international peril that killed thousands of its victims and left thousands of others permanently disfigured. The disease was so common in ancient China that newborns were not named until they survived the disease. The development of the vaccine in the late 1700s followed centuries of innovative efforts to fight smallpox.

The ancient Chinese were the first to develop an effective measure against smallpox. A snuff made from powdered smallpox scabs was blown into the nostrils of uninfected individuals. Some individuals died from the therapy; however, in most cases, the mild infection produced offered protection from later, more serious infection.

By the late 1600s, some European peasants employed a similar method of immunizing themselves against smallpox. In a practice referred to as "buying the smallpox," peasants in Poland, Scotland, and Denmark reportedly injected the smallpox virus into the skin to obtain immunity. At the time, conventional medical doctors in Europe relied solely on isolation and quarantine of people with the disease.

Changes in these practices took place, in part, through the vigorous effort of Lady **Mary Wortley Montague**, the wife of the British ambassador to Turkey in the early 1700s. Montague said the Turks injected a preparation of small pox scabs into the veins of susceptible individuals. Those injected generally developed a mild case of smallpox from which they recovered rapidly, Montague wrote.

Upon her return to Great Britain, Montague helped convince King George I to allow trials of the technique on inmates in Newgate Prison. Success of the trials cleared the way for variolation, or the direct injection of smallpox, to become accepted medical practice in England until a **vaccination** was developed later in the century. Variolation also was credited with protecting United States soldiers from smallpox during the Revolutionary War.

Regardless, doubts remained about the practice. Individuals were known to die after receiving the smallpox injections.

The next leap in the battle against smallpox occurred when **Edward Jenner** (1749–1823) acted on a hunch. Jenner observed that people who were in contact with cows often developed **cowpox**, which caused pox but was not life threatening. Those people did not develop smallpox. In 1796, Jenner decided to test his hypothesis that cowpox could be used to protect humans against smallpox. Jenner injected a healthy eight-year-old boy with cowpox obtained from a milkmaid's sore. The boy was moderately ill and recovered. Jenner then injected the boy twice with the smallpox virus, and the boy did not get sick.

Jenner's discovery launched a new era in medicine, one in which the intricacies of the immune system would become increasingly important. Contemporary knowledge suggests that cowpox was similar enough to smallpox that the **antigen** included in the vaccine stimulated an immune response to smallpox. Exposure to cowpox antigen transformed the boy's immune system, generating cells that would remember the original antigen. The smallpox vaccine, like the many others that would follow, carved a protective pattern in the immune system, one that conditioned the immune system to move faster and more efficiently against future infection by smallpox.

The term vaccination, taken from the Latin for cow (*vacca*) was developed by **Louis Pasteur** (1822–1895) a century later to define Jenner's discovery. The term also drew from the word vaccinia, the virus drawn from cowpox and developed in the laboratory for use in the smallpox vaccine. In spite of Jenner's successful report, critics questioned the wisdom of using the vaccine, with some worrying that people injected with cowpox would develop animal characteristics, such as women growing animal hair. Nonetheless, the vaccine gained popularity, and replaced the more risky direct inoculation with smallpox. In 1979, following a major cooperative effort between nations and several international organizations, world health authorities declared smallpox the only infectious disease to be completely eliminated.

The concerns expressed by Jenner's contemporaries about the side effects of vaccines would continue to follow the pioneers of vaccine development. Virtually all vaccinations continue to have side effects, with some of these effects due to the inherent nature of the vaccine, some due to the potential for impurities in a manufactured product, and some due to the potential for human error in administering the vaccine.

Virtually all vaccines would also continue to attract intense public interest. This was demonstrated in 1885 when Louis Pasteur (1822–1895) saved the life of Joseph Meister, a nine year old who had been attacked by a rabid dog. Pasteur's series of experimental **rabies** vaccinations on the boy proved the effectiveness of the new vaccine.

Until development of the rabies vaccine, Pasteur had been criticized by the public, though his great discoveries included the development of the **food preservation** process called **pasteurization**. With the discovery of a rabies vaccine, Pasteur became an honored figure. In France, his birthday declared a national holiday, and streets renamed after him.

Pasteur's rabies vaccine, the first human vaccine created in a laboratory, was made of an extract gathered from the spinal cords of rabies-infected rabbits. The live virus was weakened by drying over potash. The new vaccination was far from perfect, causing occasional fatalities and temporary paralysis. Individuals had to be injected 14–21 times.

The rabies vaccine has been refined many times. In the 1950s, a vaccine grown in duck embryos replaced the use of live virus, and in 1980, a vaccine developed in cultured human cells was produced. In 1998, the newest vaccine technology—genetically engineered vaccines—was applied to rabies. The new **DNA** vaccine cost a fraction of the regular vaccine. While

only a few people die of rabies each year in the United States, more than 40,000 die worldwide, particularly in Asia and Africa. The less expensive vaccine will make vaccination far more available to people in less developed nations.

The story of the most celebrated vaccine in modern times, the polio vaccine, is one of discovery and revision. While the **viruses** that cause polio appear to have been present for centuries, the disease emerged to an unusual extent in the early 1900s. At the peak of the epidemic, in 1952, polio killed 3,000 Americans and 58,000 new cases of polio were reported. The crippling disease caused an epidemic of fear and illness as Americans—and the world—searched for an explanation of how the disease worked and how to protect their families.

The creation of a vaccine for **poliomyelitis** by **Jonas Salk** (1914–1995) in 1955 concluded decades of a drive to find a cure. The Salk vaccine, a killed virus type, contained the three types of polio virus which had been identified in the 1940s.

In 1955, the first year the vaccine was distributed, disaster struck. Dozens of cases were reported in individuals who had received the vaccine or had contact with individuals who had been vaccinated. The culprit was an impure batch of vaccine that had not been completely inactivated. By the end of the incident, more than 200 cases had developed and 11 people had died.

Production problems with the Salk vaccine were overcome following the 1955 disaster. Then in 1961, an oral polio vaccine developed by Albert B. Sabin (1906–1993) was licensed in the United States. The continuing controversy over the virtues of the Sabin and Salk vaccines is a reminder of the many complexities in evaluating the risks versus the benefits of vaccines.

The Sabin vaccine, which used weakened, live polio virus, quickly overtook the Salk vaccine in popularity in the United States, and is currently administered to all healthy children. Because it is taken orally, the Sabin vaccine is more convenient and less expensive to administer than the Salk vaccine.

Advocates of the Salk vaccine, which is still used extensively in Canada and many other countries, contend that it is safer than the Sabin oral vaccine. No individuals have developed polio from the Salk vaccine since the 1955 incident. In contrast, the Sabin vaccine has a very small but significant rate of complications, including the development of polio. However, there has not been one new case of polio in the United States since 1975, or in the Western Hemisphere since 1991. Though polio has not been completely eradicated, there were only 144 confirmed cases worldwide in 1999.

Effective vaccines have limited many of the life-threatening infectious diseases. In the United States, children starting kindergarten are required to be immunized against polio, **diphtheria**, tetanus, and several other diseases. Other vaccinations are used only by populations at risk, individuals exposed to disease, or when exposure to a disease is likely to occur due to travel to an area where the disease is common. These include influenza, **yellow fever**, typhoid, cholera, and **Hepatitis** A and B.

The influenza virus is one of the more problematic diseases because the viruses constantly change, making development of vaccines difficult. Scientists grapple with predicting what particular influenza strain will predominate in a given year. When the prediction is accurate, the vaccine is effective. When they are not, the vaccine is often of little help.

The classic methods for producing vaccines use biological products obtained directly from a virus or a **bacteria**. Depending on the vaccination, the virus or bacteria is either used in a weakened form, as in the Sabin oral polio vaccine; killed, as in the Salk polio vaccine; or taken apart so that a piece of the microorganism can be used. For example, the vaccine for Streptococcus pneumoniae uses bacterial polysaccharides, carbohydrates found in bacteria which contain large numbers of monosaccharides, a simple sugar. These classical methods vary in safety and efficiency. In general, vaccines that use live bacterial or viral products are extremely effective when they work, but carry a greater risk of causing disease. This is most threatening to individuals whose immune systems are weakened, such as individuals with leukemia. Children with leukemia are advised not to take the oral polio vaccine because they are at greater risk of developing the disease. Vaccines which do not include a live virus or bacteria tend to be safer, but their protection may not be as great.

The classical types of vaccines are all limited in their dependence on biological products, which often must be kept cold, may have a limited life, and can be difficult to produce. The development of recombinant vaccines—those using chromosomal parts (or DNA) from a different organism—has generated hope for a new generation of man-made vaccines. The hepatitis B vaccine, one of the first recombinant vaccines to be approved for human use, is made using recombinant **yeast** cells genetically engineered to include the **gene** coding for the hepatitis B antigen. Because the vaccine contains the antigen, it is capable of stimulating **antibody** production against hepatitis B without the risk that live hepatitis B vaccine carries by introducing the virus into the blood stream.

As medical knowledge has increased—particularly in the field of DNA vaccines—researchers have set their sights on a wealth of possible new vaccines for cancer, melanoma, AIDS, influenza, and numerous others. Since 1980, many improved vaccines have been approved, including several genetically engineered (recombinant) types which first developed during an experiment in 1990. These recombinant vaccines involve the use of so-called "naked DNA." Microscopic portions of a viruses' DNA are injected into the patient. The patient's own cells then adopt that DNA, which is then duplicated when the cell divides, becoming part of each new cell. Researchers have reported success using this method in laboratory trials against influenza and **malaria**. These DNA vaccines work from inside the cell, not just from the cell's surface, as other vaccines do, allowing a stronger cell-mediated fight against the disease. Also, because the influenza virus constantly changes its surface proteins, the immune system or vaccines cannot change quickly enough to fight each new strain. However, DNA vaccines work on a core protein, which researchers believe should not be affected by these surface changes.

Since the emergence of AIDS in the early 1980s, a worldwide search against the disease has resulted in clinical trials for more than 25 experimental vaccines. These range from whole-inactivated viruses to genetically engineered types. Some have focused on a therapeutic approach to help

infected individuals to fend off further illness by stimulating components of the immune system; others have genetically engineered a protein on the surface of **HIV** to prompt immune response against the virus; and yet others attempted to protect uninfected individuals. The challenges in developing a protective vaccine include the fact that HIV appears to have multiple viral strains and mutates quickly.

In January 1999, a promising study was reported in Science magazine of a new AIDS vaccine created by injecting a healthy cell with DNA from a protein in the AIDS virus that is involved in the infection process. This cell was then injected with genetic material from cells involved in the immune response. Once injected into the individual, this vaccine "catches the AIDS virus in the act," exposing it to the immune system and triggering an immune response. This discovery offers considerable hope for development of an effective vaccine. As of June 2002, a proven vaccine for AIDS had not yet been proven in clinical trials.

Stimulating the immune system is also considered key by many researchers seeking a vaccine for cancer. Currently numerous clinical trials for cancer vaccines are in progress, with researchers developing experimental vaccines against cancer of the breast, colon, and lung, among other areas. Promising studies of vaccines made from the patient's own tumor cells and genetically engineered vaccines have been reported. Other experimental techniques attempt to penetrate the body in ways that could stimulate vigorous immune responses. These include using bacteria or viruses, both known to be efficient travelers in the body, as carriers of vaccine antigens. Such bacteria or viruses would be treated or engineered to make them incapable of causing illness.

Current research also focuses on developing better vaccines. The Children's Vaccine Initiative, supported by the **World Health Organization**, the United Nation's Children's Fund, and other organizations, are working diligently to make vaccines easier to distribute in developing countries. Although more than 80% of the world's children were immunized by 1990, no new vaccines have been introduced extensively since then. More than four million people, mostly children, die needlessly every year from preventable diseases. Annually, measles kills 1.1 million children worldwide; whooping cough (**pertussis**) kills 350,000; hepatitis B 800,000; **Haemophilus** influenzae type b (Hib) 500,000; tetanus 500,000; rubella 300,000; and yellow fever 30,000. Another 8 million die from diseases for which vaccines are still being developed. These include pneumococcal **pneumonia** (1.2 million); acute respiratory virus infections (400,000), malaria (2 million); AIDS (2.3 million); and rotavirus (800,000). In August, 1998, the Food and Drug Administration approved the first vaccine to prevent rotavirus—a severe diarrhea and vomiting infection.

The measles epidemic of 1989 was a graphic display of the failure of many Americans to be properly immunized. A total of 18,000 people were infected, including 41 children who died after developing measles, an infectious, viral illness whose complications include pneumonia and encephalitis. The epidemic was particularly troubling because an effective, safe vaccine against measles has been widely distributed in the United States since the late 1960s. By 1991, the number of new measles cases had started to decrease, but health officials warned that measles remained a threat.

Vaccines stimulate the production of antibodies that provide immunity from disease.

This outbreak reflected the limited reach of vaccination programs. Only 15% of the children between the ages of 16 and 59 months who developed measles between 1989 and 1991 had received the recommended measles vaccination. In many cases parent's erroneously reasoned that they could avoid even the minimal risk of vaccine side effects "because all other children were vaccinated."

Nearly all children are immunized properly by the time they start school. However, very young children are far less likely to receive the proper vaccinations. Problems behind the lack of **immunization** range from the limited health care received by many Americans to the increasing cost of vaccinations. Health experts also contend that keeping up with a vaccine schedule, which requires repeated visits, may be too challenging for Americans who do not have a regular doctor or health provider.

Internationally, the challenge of vaccinating large numbers of people has also proven to be immense. Also, the reluctance of some parents to vaccinate their children due to potential side effects has limited vaccination use. Parents in

the United States and several European countries have balked at vaccinating their children with the pertussis vaccine due to the development of neurological complications in a small number of children given the vaccine. Because of incomplete immunization, whooping cough remains common in the United States, with 30,000 cases and about 25 deaths due to complications annually. One response to such concerns has been testing in the United States of a new pertussis vaccine that has fewer side effects.

Researchers look to genetic engineering, gene discovery, and other innovative technologies to produce new vaccines.

See also AIDS, recent advances in research and treatment; Antibody formation and kinetics; Bacteria and bacterial infection; Bioterrorism, protective measures; Immune stimulation, as a vaccine; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunochemistry; Immunogenetics; Immunologic therapies; Immunology; Interferon actions; Poliomyelitis and polio; Smallpox, eradication, storage, and potential use as a bacteriological weapon

VARICELLA

Varicella, commonly known as chickenpox, is a disease characterized by skin lesions and low-grade fever, and is common in the United States and other countries located in areas with temperate climates. The incidence of varicella is extremely high; almost everyone living in the United States is exposed to the disease, usually during childhood, but sometimes in adulthood. In the United States, about 3.9 million people a year contract varicella. A highly contagious disease, varicella is caused by Varicella-Zoster virus (VZV), the same virus that causes the skin disease shingles. For most cases of varicella, no treatment besides comfort measures and management of itching and fever is necessary. In some cases, however, varicella may evolve into more serious conditions, such as **bacterial infection** of the skin lesions or **pneumonia**. These complications tend to occur in persons with weakened immune systems, such as children receiving **chemotherapy** for cancer, or people with Acquired Immune Deficiency Syndrome (**AIDS**). A **vaccine** for varicella is now receiving widespread use.

There are two possible origins for the colloquialism "chickenpox." Some think that "chicken" comes from the French word chiche (chick-pea) because at one stage of the disease, the lesions may resemble chick-peas. Others think that "chicken" may have evolved from the Old English word gigan (to itch). Interestingly, the term "varicella" is a diminutive form of the term "variola," the Latin word for **smallpox**. Although both varicella and smallpox are viral diseases that cause skin lesions, smallpox is more deadly and its lesions cause severe scarring.

Varicella is spread by breathing in respiratory droplets spread through the air by a cough or sneeze of an infected individual. Contact with the fluid from skin lesions can also spread the virus. The incubation period, or the time from exposure to VZV to the onset of the disease, is about 14–15 days. The most contagious period is just prior to the appearance of the rash, and early in the illness, when fresh vesicles are still appearing. The first sign of varicella in children is often the appearance of the varicella rash. Adults and some children may have a prodrome, or series of warning symptoms. This prodrome is typical of the flu, and includes headache, fatigue, backache, and a fever. The onset of the rash is quite rapid. First, a diffuse, small, red dot-like rash appears on the skin. Soon, a vesicle containing clear fluid appears in the center of the dots. The vesicle rapidly dries, forming a crust. This cycle, from the appearance of the dot to the formation of the crust, can take place within eight to 12 hours. As the crust dries, it falls off, leaving a slight depression that eventually recedes. Significant scarring from varicella is rare.

Over the course of a case of varicella, an individual may develop between 250 and 500 skin lesions. The lesions occur in waves, with the first set of lesions drying up just as successive waves appear. The waves appear over two to four days. The entire disease runs its course in about a week, but the lesions continue to heal for about two to three weeks. The lesions first appear on the scalp and trunk. Most of the lesions in varicella are found at the center of the body; few lesions form on the soles and palms. Lesions are also found on the mucous membranes, such as the respiratory tract, the gastrointestinal tract, and the urogenital tract. Researchers think that the lesions on the respiratory tract may help transmit the disease. If a person with respiratory lesions coughs, they may spray some of the vesicle fluid into the atmosphere, to be breathed by other susceptible persons.

Although the lesions may appear alarming, varicella in children is usually a mild disease with few complications and a low fever. Occasionally, if the rash is severe, the fever may be higher. Varicells is more serious in adults, who usually have a higher fever and general malaise. The most common complaint about varicella from both children and adults is the itching caused by the lesions. It is important not to scratch the lesions, as scratching may cause scarring.

Because varicella is usually a mild disease, no drug treatment is normally prescribed. For pain or fever relief associated with varicella, physicians recommended avoiding salicylate, or aspirin. Salicylate may contribute to Reye's syndrome, a serious neurological condition that is especially associated with aspirin intake and varicella; in fact, 20–30% of the total cases of Reye's syndrome occur in children with varicella.

Varicella, although not deadly for most people, can be quite serious in those who have weakened immune systems, and drug therapy is recommended for these cases. **Antiviral drugs** (such as acyclovir) have been shown to lessen the severity and duration of the disease, although some of the side effects, such as gastrointestinal upset, can be problematic.

If the lesions are severe and the person has scratched them, bacterial infection of the lesions can result. This complication is managed with antibiotic treatment. A more serious complication is pneumonia. Pneumonia is rare in otherwise healthy children and is more often seen in older patients or in children who already have a serious disease, such as cancer. Pneumonia is also treated with **antibiotics**. Another complica-

tion of varicella is shingles. Shingles are painful outbreaks of skin lesions that occur some years after a bout with varicella. Shingles are caused by VZV left behind in the body that eventually reactivates. Shingles causes skin lesions and burning pain along the region served by a specific nerve. It is not clear why VZV is reactivated in some people and not in others, but many people with compromised immune systems can develop severe, even life-threatening cases of shingles.

Pregnant women are more susceptible to varicella, which also poses a threat to both prenatal and newborn children. If a woman contracts varicella in the first trimester (first three months) of pregnancy, the fetus may be at increased risk for birth defects such as eye damage. A newborn may contract varicella in the uterus if the mother has varicella five days before birth. Newborns can also contract varicella if the mother has the disease up to two days after birth. Varicella can be a deadly disease for newborns; the fatality rate from varicella in newborns up to five days old approaches 30%. For this reason, women contemplating pregnancy may opt to be vaccinated with the new VZV vaccine prior to conception if they have never had the disease. If this has not been done, and a pregnant woman contracts varicella, an injection of varicella-zoster immunoglobulin can lessen the chance of complications to the fetus.

Researchers have long noted the seasonality of varicella. According to their research, varicella cases occur at their lowest rate during September. Numbers of cases increase throughout the autumn, peak in March and April, and then fall sharply once summer begins. This cycle corresponds to the typical school year in the United States. When children go back to school in the fall, they begin to spread the disease; when summer comes and school ends, cases of varicella diminish. Varicella can spread quickly within a school when one child contracts varicella. This child rapidly infects other susceptible children. Soon, all the children who had not had varicella contract the disease within two or three cycles of transmission. It is not uncommon for high numbers of children to be infected during a localized outbreak; one school with 69 children reported that the disease struck 67 of these students.

Contrary to popular belief, it is possible to get varicella a second time. If a person had a mild case during childhood, his or her **immunity** to the virus may be weaker than that of someone who had a severe childhood case. In order to prevent varicella, especially in already-ill children and immunocompromised patients, researchers have devised a VZV vaccine, consisting of live, attenuated (modified) VZV. **Immunization** recommendations of the American Academy of Pediatrics state that children between 12 and 18 months of age who have not yet had varicella should receive the vaccine. Immunization can be accomplished with a single dose. Children up to the age of 13 who have had neither varicella nor the immunization, should also receive a single dose of the vaccine. Children older than age 13 who have never had either varicella or the vaccine should be immunized with two separate doses, given about a month apart. The vaccine provokes immunity against the virus. Although some side effects have been noted, including a mild rash and the reactivation of shingles, the vaccine is considered safe and effective.

See also Immunity, active, passive and delayed; Immunity, cell mediated; Viruses and responses to viral infection

VARICELLA ZOSTER VIRUS

Varicella zoster virus is a member of the alphaherpesvirus group and is the cause of both chickenpox (also known as varicella) and shingles (**herpes** zoster).

The virus is surrounded by a covering, or envelope, that is made of lipid. As such, the envelope dissolves readily in solvents such as alcohol. Wiping surfaces with alcohol is thus an effective means of inactivating the virus and preventing spread of chickenpox. Inside the lipid envelope is a protein shell that houses the **deoxyribonucleic acid**.

Varicella zoster virus is related to Herpes Simplex **viruses** types 1 and 2. Indeed, nucleic acid analysis has revealed that the genetic material of the three viruses is highly similar, both in the genes present and in the arrangement of the genes.

Chickenpox is the result of a person's first infection with the virus. Typically, chickenpox occurs most often in children. From 75% to 90% of the cases of chickenpox occur in children under five years old. Acquisition of the virus is usually via inhalation of droplets containing the virus. From the lung the virus migrates to the blood stream. Initially a sore throat leads to a blister-like rash that appears on the skin and the mucous membranes, as the virus is carried through the blood stream to the skin. The extent of the rash varies, from minimal to all over the body. The latter is also accompanied by fever, itching, abdominal pain, and a general feeling of tiredness. Recovery is usually complete within a week or two and **immunity** to another bout of chickenpox is life-long.

In terms of a health threat, childhood chickenpox is advantageous. The life-long immunity conferred to the child prevents adult onset infections that are generally more severe. However, chickenpox can be dangerous in infants, whose immune systems are undeveloped. Also chickenpox carries the threat of the development of sudden and dangerous liver and brain damage. This condition, called Reye's Syndrome, seems related to the use of aspirin to combat the fever associated with chickenpox (as well as other childhood viruses). When adults acquire chickenpox, the symptoms can be much more severe than those experienced by a child. In immunocompromised people, or those suffering from leukemia, chickenpox can be fatal. The disease can be problematic in pregnant women in terms of birth defects and the development of **pneumonia**.

Treatment for chickenpox is available. A drug called acyclovir can slow the replication of the virus. Topical lotions can ease the itching associated with the disease. However, in mild to moderate cases, intervention is unnecessary, other than keeping the affected person comfortable. The life-long immunity conferred by a bout of chickenpox is worth the temporary inconvenience of the malady. The situation is different for adults. Fortunately for adults, a **vaccine** to chickenpox exists for those who have not contracted chickenpox in their childhood.

Naturally acquired immunity to chickenpox does not prevent individuals from contracting shingles years, even decades later. Shingles occurs in between 10% and 20% of those who have had chickenpox. In the United States, upwards of 800,000 people are afflicted with shingles each year. The annual number of shingles sufferers worldwide is in the millions. The disease occurs most commonly in those who are over 50 years of age.

As the symptoms of chickenpox fade, varicella zoster virus is not eliminated from the body. Rather, the virus lies dormant in nerve tissue, particularly in the face and the body. The roots of sensory nerves in the spinal cord are also a site of virus hibernation. The virus is stirred to replicate by triggers that are as yet unclear. Impairment of the **immune system** seems to be involved, whether from **immunodeficiency diseases** or from cancers, the effect of drugs, or a generalized debilitation of the body with age. Whatever forces of the immune system that normally operate to hold the hibernating virus in check are abrogated.

Reactivation of the virus causes pain and a rash in the region that is served by the affected nerves. The affected areas are referred to as dermatomes. These areas appear as a rash or blistering of the skin. This can be quite painful during the one to two weeks they persist. Other complications can develop. For example, shingles on the face can lead to an eye infection causing temporary or even permanent blindness. A condition of muscle weakness or paralysis, known as Guillan-Barre Syndrome, can last for months after a bout of shingles. Another condition known as postherpetic neuralgia can extend the pain of shingles long after the visible symptoms have abated.

See also Immunity, active, passive and delayed; Infection and resistance; Latent viruses and diseases

VARIOLA VIRUS

Variola virus (or variola major virus) is the virus that causes **smallpox**. The virus is one of the members of the poxvirus group (Family *Poxviridae*). The virus particle is brick shaped and contains a double strand of **deoxyribonucleic acid**. The variola virus is among the most dangerous of all the potential biological weapons.

Variola virus infects only humans. The virus can be easily transmitted from person to person via the air. Inhalation of only a few virus particles is sufficient to establish an infection. Transmission of the virus is also possible if items such as contaminated linen are handled. The various common symptoms of smallpox include chills, high fever, extreme tiredness, headache, backache, vomiting, sore throat with a cough, and sores on mucus membranes and on the skin. As the sores burst and release pus, the afflicted person can experience great pain. Males and females of all ages are equally susceptible to infection. At the time of smallpox eradication approximately one third of patients died—usually within a period of two to three weeks following appearance of symptoms.

The origin of the variola virus in not clear. However, the similarity of the virus and **cowpox** virus has prompted the suggestion that the variola virus is a mutated version of the cowpox virus. The mutation allowed to virus to infect humans. If such a mutation did occur, then the adoption of farming activities by people, instead of the formally nomadic existence, would have been a selective pressure for a virus to adopt the capability to infect humans.

Vaccination to prevent infection with the variola virus is long established. In the 1700s, English socialite and **public health** advocate Lady **Mary Wortley Montague** popularized the practice of injection with the pus obtained from smallpox sores as a protection against the disease. This technique became known as variolation. Late in the same century, **Edward Jenner** successfully prevented the occurrence of smallpox by an injection of pus from cowpox sores. This represented the start of vaccination.

Vaccination has been very successful in dealing with variola virus outbreaks of smallpox. Indeed, after two decades of worldwide vaccination programs, the virus has been virtually eliminated from the natural environment. The last recorded case of smallpox infection was in 1977 and vaccination against smallpox is not practiced anymore.

In the late 1990s, a resolution was passed at the World Health Assembly that the remaining stocks of variola virus be destroyed, to prevent the re-emergence of smallpox and the misuse of the virus as a biological weapon. At the time only two high-security laboratories were thought to contain variola virus stock (**Centers for Disease Control** and Prevention in Atlanta, Georgia, and the Russian State Centre for Research on **Virology** and **Biotechnology**, Koltsovo, Russia). However, this decision was postponed until 2002, and now the United States government has indicated its unwillingness to comply with the resolution for security issues related to potential **bioterrorism**. Destruction of the stocks of variola virus would deprive countries of the material needed to prepare **vaccine** in the event of the deliberate use of the virus as a biological weapon. This scenario has gained more credence in the past decade, as terrorist groups have demonstrated the resolve to use biological weapons, including smallpox. In addition, intelligence agencies in several Western European countries issued opinions that additional stocks of the variola virus exist in other than the previously authorized locations.

See also Bioterrorism, protective measures; Bioterrorism; Centers for Disease Control (CDC); Smallpox, eradication, storage, and potential use as a bacteriological weapon; Viral genetics; Virology; Virus replication; Viruses and responses to viral infection

VENTER, JOHN CRAIG (1946-)

American molecular biologist

John Craig Venter, who until January 2002 was the President and Chief Executive Officer of Celera Genomics, is one of the central figures in the Human Genome Project. Venter cofounded Celera in 1998, and he directed its research and operations while he and the company's other scientists completed a draft of the human genome. Using a fast sequencing tech-

nique, Venter and his colleagues were able to sequence the human genome, and the genomes of other organisms, including the bacterium *Haemophilus infuenzae,*.

Venter was born in Salt Lake City, Utah. After high school he seemed destined for a career as a surfer rather than as a molecular biologist. But a tour of duty in Vietnam as a hospital corpsman precipitated a change in the direction of his life. He returned from Vietnam and entered university, earning a doctorate in physiology and pharmacology from the University of California at San Diego. After graduation he took a research position at the National Institutes of Health. While at NIH, Venter became frustrated at the then slow pace of identifying and sequencing genes. He began to utilize a technology that decodes only a portion of the **DNA** from normal copies of genes made by living cells. These partial transcripts, called expressed sequence tags, could then be used to identify the gene-coding regions on the DNA from which they came. The result was to speed up the identification of genes. Hundreds of genes could be discovered in only weeks using the method.

Supported by venture capital, Venter started a nonprofit company called **The Institute for Genomic Research** (**TIGR**) in the mid-1990s. TIGR produced thousands of the expressed sequence tag probes to the human genome.

Venter's success and technical insight attracted the interest of PE Biosystems, makers of automated DNA sequencers. With financial and equipment backing from PE Biosystems, Venter left TIGR and formed a private for-profit company, Celera (meaning 'swift' in Latin). The aim was to decode the human genome faster than the government effort that was underway. Celera commenced operations in May 1998.

Another of Venter's accomplishments was to use a nontraditional approach to quickly sequence DNA. At that time, DNA was typically sequenced by dividing it into several large pieces and then decoding each piece. Venter devised the so-called shotgun method, in which a genome was blown apart into many small bits and then to sequence them without regard to their position. Following sequencing, supercomputer power would reassemble the bits of sequence into the intact genome sequence. The technique, which was extremely controversial, was tried first on the genome of the fruit fly *Drosophila*. In only a year the fruit fly genome sequence was obtained. The sequencing of the genome of the bacterium *H. influenzae* followed this.

Although the privatization of human genome sequence data remains highly controversial, Venter's accomplishments are considerable, both technically and as a force within the scientific community to spur genome sequencing.

See also DNA (Deoxyribonucleic acid); DNA hybridization; Economic uses and benefits of microorganisms; Genetic code; Genetic identification of microorganisms; Genetic mapping; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Genotype and phenotype; Immunogenetics; Molecular biology and molecular genetics

VETERINARY MICROBIOLOGY

Veterinary microbiology is concerned with the **microorganisms**, both beneficial and disease causing, to non-human animal life. For a small animal veterinarian, the typical animals of concern are domesticated animals, such as dogs, cats, birds, fish, and reptiles. Large animal veterinarians focus on animals of economic importance, such as horses, cows, sheep, and poultry.

The dogs and cats that are such a familiar part of the household environment are subject to a variety of microbiological origin ailments. As with humans, **vaccination** of young dogs and cats is a wise precaution to avoid microbiological diseases later in life.

Cats can be infected by a number of **viruses** and **bacteria** that cause respiratory tract infections. For example the bacterium *Bordetella pertussis*, the common cause of kennel cough in dogs, also infects cats, causing the same persistent cough. Another bacteria called *Chlamydia* causes another respiratory disease, although most of the symptoms are apparent in the eyes. **Inflammation** of the mucous covering of the eyelids (conjunctivitis) can be so severe that the eyes swell shut.

Cats are prone to viral infections. Coronavirus is common in environments such as animal shelters, where numbers of cats live in close quarters. The virus causes an infection of the intestinal tract. Feline panleukopenia is a very contagious viral disease that causes a malaise and a decrease in the number of white blood cells. The immune disruption can leave the cat vulnerable to other infections and can be lethal. Fortunately, a protective **vaccine** exists. Like humans, cats are also prone to **herpes** virus infections. In cats the infection is in the respiratory tract and eyes. Severe infections can produce blindness. Another respiratory disease, reminiscent of a **cold** in humans, is caused by a calicivirus. **Pneumonia** can develop and is frequently lethal. Finally the feline leukemia virus causes cancer of the blood. The highly contagious nature of this virus makes vaccination prudent for young kittens.

Dogs are likewise susceptible to bacterial and viral infections. A virus known as parainfluenzae virus also causes kennel cough. Dogs are also susceptible to coronavirus. Members of the bacterial genus called *Leptospira* can infect the kidneys. This infection can be passed to humans and to other animals. A very contagious viral infection, which typically accompanies bacterial infections, is called canine distemper. Distemper attacks many organs in the body and can leave the survivor permanently disabled. A vaccine against distemper exists, but must be administered periodically throughout the dog's life to maintain the protection. Another virus called parvovirus produces a highly contagious, often fatal, infection. Once again, vaccination needs to be at regular (usually yearly) intervals. Like humans, dogs are susceptible to **hepatitis**, a destructive viral disease of the liver. In dogs that have not been vaccinated, the liver infection can be debilitating. Finally, dogs are also susceptible to the viral agent of **rabies**. The virus, often passed to the dog via the bite of another rabid animal, can in turn be passed onto humans. Fortunately again, vaccination can eliminated the risk of acquiring rabies.

Two veterinarians treat a dog with an infected leg.

Microbiological infections of farm animals and poultry is common. For example, studies have shown that well over half the poultry entering processing plants are infected with the bacterium *Campylobacter jejuni*. Infection with members of the bacterial genus *Salmonella* are almost as common. Fecal **contamination** of poultry held in close quarters is responsible. Similarly the intestinal bacterium ***Escherichia coli*** is spread from bird to bird. Improper processing can pass on these bacteria to humans, where they cause intestinal maladies.

Chickens and turkeys are also susceptible to a bacterial respiratory disease caused by *Mycoplasma spp*. The "air sac disease" causes lethargy, weight loss, and decreased egg production. Poultry can also acquire a form of cholera, which is caused by *Pasteurella multocida*. Examples of some other bacteria of note in poultry are species of *Clostridium* (intestinal tract infection and destruction of tissue), *Salmonella pullorum* (intestinal infection that disseminates widely throughout the body), *Salmonella gallinarum* (typhoid), and *Clostridium botulinum* (**botulism**).

Cattle and sheep are also susceptible to microbiological ailments. Foot and mouth disease is a prominent example. This contagious and fatal disease can sweep through cattle and sheep populations, causing financial ruin for ranchers. Moreover, there is now evidence that bovine spongiform encephalopathy, a disease caused by an infectious agent termed a prion, may be transmissible to humans, where it is manifest as the always lethal brain deterioration called Creutzfeld-Jacob disease.

See also Zoonoses

VIABLE BUT NONCULTURABLE BACTERIA

Viable but nonculturable **bacteria** are bacteria that are alive, but which are not growing or dividing. Their metabolic activity is almost nonexistent.

This state was recognized initially by microbial ecologists examining bacterial populations in natural sediments. Measurements of the total bacterial count, which counts both living and dead bacteria, are often far higher than the count of the living bacteria. At certain times of year, generally when nutrients are plentiful, the total and living numbers match more closely. These observations are not the result of seasonal "die-off," but reflect the adoption of an almost dormant mode of existence by a sizable proportion of some bacterial populations.

A viable but nonculturable bacteria cannot be cultured on conventional laboratory growth media but can be demonstrated to be alive by other means, such as the uptake and **metabolism** of radioactively labeled nutrients. Additionally, the microscopic examination of populations shows the bacteria to be intact. When bacteria die they often lyse, due to the release of **enzymes** that disrupt the interior and the cell wall of the bacteria.

The viable but nonculturable state is reversible. Bacterial that do not form spores can enter the state when conditions become lethal for their continued growth. The state is a means of bacterial survival to stresses that include elevated salt concentration, depletion of nutrients, depletion of oxygen, and exposure to certain wavelengths of light. When the stress is removed, bacteria can revive and resume normal growth.

The shift to the nonculturable state triggers the expression of some 40 genes in bacteria. As well, the composition of the cell wall changes, becoming enhanced in fatty acid constituents, and the genetic material becomes coiled more tightly.

The entry of a bacterium into the nonculturable state varies from days to months. Younger bacterial cells are capable of a more rapid transition than are older cells. In general, however, the transition to a nonculturable state seems to be in response to a more gradual change in the environment than other bacterial stress responses, (e.g., spore formation, **heat shock response**).

In contrast to the prolonged entry into the quiescent phase, the exit from the viable but nonculturable state is quite rapid (within hours for *Vibrio vulnifucus*). Other bacteria, such as *Legionella pneumophila*, the causative agent of Legionnaires' disease, revive much more slowly. The adoption of this mode of survival by disease-causing bacteria further complicates strategies to detect and eradicate them.

See also Bacteria and bacterial infection; Bacterial adaptation

VIRAL EPIDEMICS • *see* EPIDEMICS, VIRAL

VIRAL GENETICS

Viral genetics, the study of the genetic mechanisms that operate during the life cycle of **viruses**, utilizes biophysical, biological, and genetic analyses to study the viral genome and its variation. The virus genome consists of only one type of nucleic acid, which could be a single or double stranded **DNA** or **RNA**. Single stranded RNA viruses could contain positive-sense (+RNA), which serves directly as mRNA or negative-sense RNA (–RNA) that must use an RNA polymerase to synthesize a complementary positive strand to serve as mRNA. Viruses are obligate **parasites** that are completely dependant on the host cell for the replication and **transcription** of their genomes as well as the **translation** of the mRNA transcripts into proteins. Viral proteins usually have a structural function, making up a shell around the genome, but may contain some **enzymes** that are necessary for the **virus replication** and life cycle in the host cell. Both bacterial virus (bacteriophages) and animal viruses play an important role as tools in molecular and cellular biology research.

Viruses are classified in two families depending on whether they have RNA or DNA genomes and whether these genomes are double or single stranded. Further subdivision into types takes into account whether the genome consists of a single RNA molecule or many molecules as in the case of segmented viruses. Four types of bacteriophages are widely used in biochemical and genetic research. These are the T phages, the temperate phages typified by **bacteriophage** lambda, the small DNA phages like M13, and the RNA phages. Animal viruses are subdivided in many classes and types. Class I viruses contain a single molecule of double stranded DNA and are exemplified by adenovirus, simian virus 40 (SV40), **herpes** viruses and human papilloma viruses. Class II viruses are also called parvoviruses and are made of single stranded DNA that is copied in to double stranded DNA before transcription in the host cell. Class III viruses are double stranded RNA viruses that have segmented genomes which means that they contain 10–12 separate double stranded RNA molecules. The negative strands serve as template for mRNA synthesis. Class IV viruses, typified by poliovirus, have single plus strand genomic RNA that serves as the mRNA. Class V viruses contain a single negative strand RNA which serves as the template for the production of mRNA by specific virus enzymes. Class VI viruses are also known as **retroviruses** and contain double stranded RNA genome. These viruses have an enzyme called reverse transcriptase that can both copy minus strand DNA from genomic RNA catalyze the synthesis of a complementary plus DNA strand. The resulting double stranded DNA is integrated in the host chromosome and

is transcribed by the host own machinery. The resulting transcripts are either used to synthesize proteins or produce new viral particles. These new viruses are released by budding, usually without killing the host cell. Both **HIV** and **HTLV** viruses belong to this class of viruses.

Virus genetics are studied by either investigating genome **mutations** or exchange of genetic material during the life cycle of the virus. The frequency and types of genetic variations in the virus are influenced by the nature of the viral genome and its structure. Especially important are the type of the nucleic acid that influence the potential for the viral genome to integrate in the host, and the segmentation that influence exchange of genetic information through assortment and **recombination**.

Mutations in the virus genome could either occur spontaneously or be induced by physical and chemical means. Spontaneous mutations that arise naturally as a result of viral replication are either due to a defect in the genome replication machinery or to the incorporation of an analogous base instead of the normal one. Induced virus **mutants** are obtained by either using chemical mutants like nitrous oxide that acts directly on bases and modify them or by incorporating already modified bases in the virus genome by adding these bases as substrates during virus replication. Physical agents such as ultra-violet light and x rays can also be used in inducing mutations. Genotypically, the induced mutations are usually point mutations, deletions, and rarely insertions. The **phenotype** of the induced mutants is usually varied. Some mutants are conditional lethal mutants. These could differ from the wild type virus by being sensitive to high or low temperature. A low temperature mutant would for example grow at 88°F (31°C) but not at 100°F (38°C), while the wild type will grow at both temperatures. A mutant could also be obtained that grows better at elevated temperatures than the wild type virus. These mutants are called hot mutants and may be more dangerous for the host because fever, which usually slows the growth of wild type virus, is ineffective in controlling them. Other mutants that are usually generated are those that show drug resistance, enzyme deficiency, or an altered pathogenicity or host range. Some of these mutants cause milder symptoms compared to the parental virulent virus and usually have potential in **vaccine** development as exemplified by some types of **influenza** vaccines.

Besides mutation, new genetic variants of viruses also arise through exchange of genetic material by recombination and reassortment. Classical recombination involves the breaking of covalent bonds within the virus nucleic acid and exchange of some DNA segments followed by rejoining of the DNA break. This type of recombination is almost exclusively reserved to DNA viruses and retroviruses. RNA viruses that do not have a DNA phase rarely use this mechanism. Recombination usually enables a virus to pick up genetic material from similar viruses and even from unrelated viruses and the eukaryotic host cells. Exchange of genetic material with the host is especially common with retroviruses. Reassortment is a non-classical kind of recombination that occurs if two variants of a segmented virus infect the same cell. The resulting progeny virions may get some segments from one parent and some from the other. All known segmented virus that infect humans are RNA viruses. The process of reassortment is very efficient in the exchange of genetic material and is used in the generation of viral vaccines especially in the case of influenza live vaccines. The ability of viruses to exchange genetic information through recombination is the basis for virus-based vectors in recombinant DNA technology and hold great promises in the development of **gene** therapy. Viruses are attractive as vectors in gene therapy because they can be targeted to specific tissues in the organs that the virus usually infect and because viruses do not need special chemical reagents called transfectants that are used to target a plasmid vector to the genome of the host.

Genetic variants generated through mutations, recombination or reassortment could interact with each other if they infected the same host cell and prevent the appearance of any **phenotype**. This phenomenon, where each mutant provide the missing function of the other while both are still genotypically mutant, is known as complementation. It is used as an efficient tool to determine if mutations are in unique or in different genes and to reveal the minimum number of genes affecting a function. Temperature sensitive mutants that have the same mutation in the same gene will for example not be able to **complement** each other. It is important to distinguish complementation from multiplicity reactivation where a higher dose of inactivated mutants will be reactivated and infect a cell because these inactivated viruses cooperate in a poorly understood process. This reactivation probably involves both a complementation step that allows defective viruses to replicate and a recombination step resulting in new genotypes and sometimes regeneration of the wild type. The viruses that need complementation to achieve an infectious cycle are usually referred to as defective mutants and the complementing virus is the helper virus. In some cases, the defective virus may interfere with and reduce the infectivity of the helper virus by competing with it for some factors that are involved in the viral life cycle. These defective viruses called "defective interfering" are sometimes involved in modulating natural infections. Different wild type viruses that infect the same cell may exchange coat components without any exchange of genetic material. This phenomenon, known as phenotypic mixing is usually restricted to related viruses and may change both the morphology of the packaged virus and the tropism or tissue specificity of these infectious agents.

See also Viral vectors in gene therapy; Virology; Virus replication; Viruses and responses to viral infection

VIRAL INFECTIONS • *see* VIRUSES AND RESPONSES TO VIRAL INFECTION

VIRAL VECTORS IN GENE THERAPY

Gene therapy is the introduction of a gene into cells to reverse a functional defect caused by a defect in a host genome (the set of genes present in an organism).

The use of **viruses** quickly became an attractive possibility once the possibility of gene therapy became apparent. Viruses require other cells for their replication. Indeed, an essential feature of a **virus replication** cycle is the transfer of their genetic material (**deoxyribonucleic acid**, **DNA**; or **ribonucleic acid**, **RNA**) into the host cell, and the replication of that material in the host cell. By incorporating other DNA or RNA into the virus genome, the virus then becomes a vector for the transmission of that additional genetic material. Finally, if the inserted genetic material is the same as a sequence in the host cell that is defective, then the expression of the inserted gene will provide the product that the defective host genome does not. As a result, host defective host genetic function and the consequences of the defects can be reduced or corrected.

Retroviruses contain RNA as the genetic material. A viral enzyme called reverse transcriptase functions to manufacture DNA from the RNA, and the DNA can then become incorporated into the host DNA. Despite the known involvement of some retroviruses in cancer, these viruses are attractive for gene therapy because of their pronounced tendency to integrate the viral DNA into the host genome. Retroviruses used as gene vectors also have had the potential cancer-causing genetic information deleted. The most common retrovirus that has been used in experimental gene therapy is the Moloney murine leukaemia virus. This virus can infect cells of both mice and humans. This makes the results obtained from mouse studies more relevant to humans.

Adenoviruses are another potential gene vector. Once they have infected the host cell, many rounds of DNA replication can occur. This is advantageous, as much of the therapeutic product could be produced. However, because integration of the virally transported gene does not occur, the expression of the gene only occurs for a relatively short time. To produce levels of the gene product that would have a substantial effect on a patient, the virus vector needs to administered repeatedly. As for retroviruses, the adenoviruses used as vectors need to be crippled so as to prevent the production of new viruses.

Adenovirus vector has been used to correct **mutations** the gene that is defective in cystic fibrosis. However, as of May 2002, the success rate in human trials remained low. In addition, the immune response to the high levels of the vector that are needed can be problematic.

Another important aspect of gene therapy concerns the target of the viral vectors. The viruses need to be targeted at host cells that are actively dividing, because only in cells in which DNA replication is occurring will the inserted viral genetic material be replicated. This is one reason why cancers are a conceptually attractive target of virus-mediated gene therapy, as cancerous cells are dangerous by virtue of their rapid and uncontrolled division.

Cancerous cells arise by some form of mutation. Therefore, therapy to replace defective genes with functional genes holds promise for cancer researchers. The target of gene therapy can vary, as many cancers have mutations that direct a normal cell towards acquiring the potential to become cancerous, and other mutations that inactivate mechanisms that function to regulate growth control. Furthermore, gene therapy can be directed at the **immune system** rather than directly at the cancerous cell. An example of this strategy is known as immunopotentiation (the enhancement of the immune response to cancers).

A risk of viral gene therapy, in those viruses that operate by integrating genetic material into the host genome, is the possibility of damage to the host DNA by the insertion. Alteration of some other host gene could have unforeseen and undesirable side effects. The elimination of this possibility will require further technical refinements. Adenoviruses are advantageous in this regard as the replication of their DNA in the host cell does not involve insertion of the viral DNA into the host DNA. Accordingly, the possibility of mutations due to insertion do not exist.

The September 1999 death of an 18 year old patient with a rare metabolic condition, who died while receiving viral gene therapy, considerably slowed progress on clinical applications of viral gene therapy.

See also Biotechnology

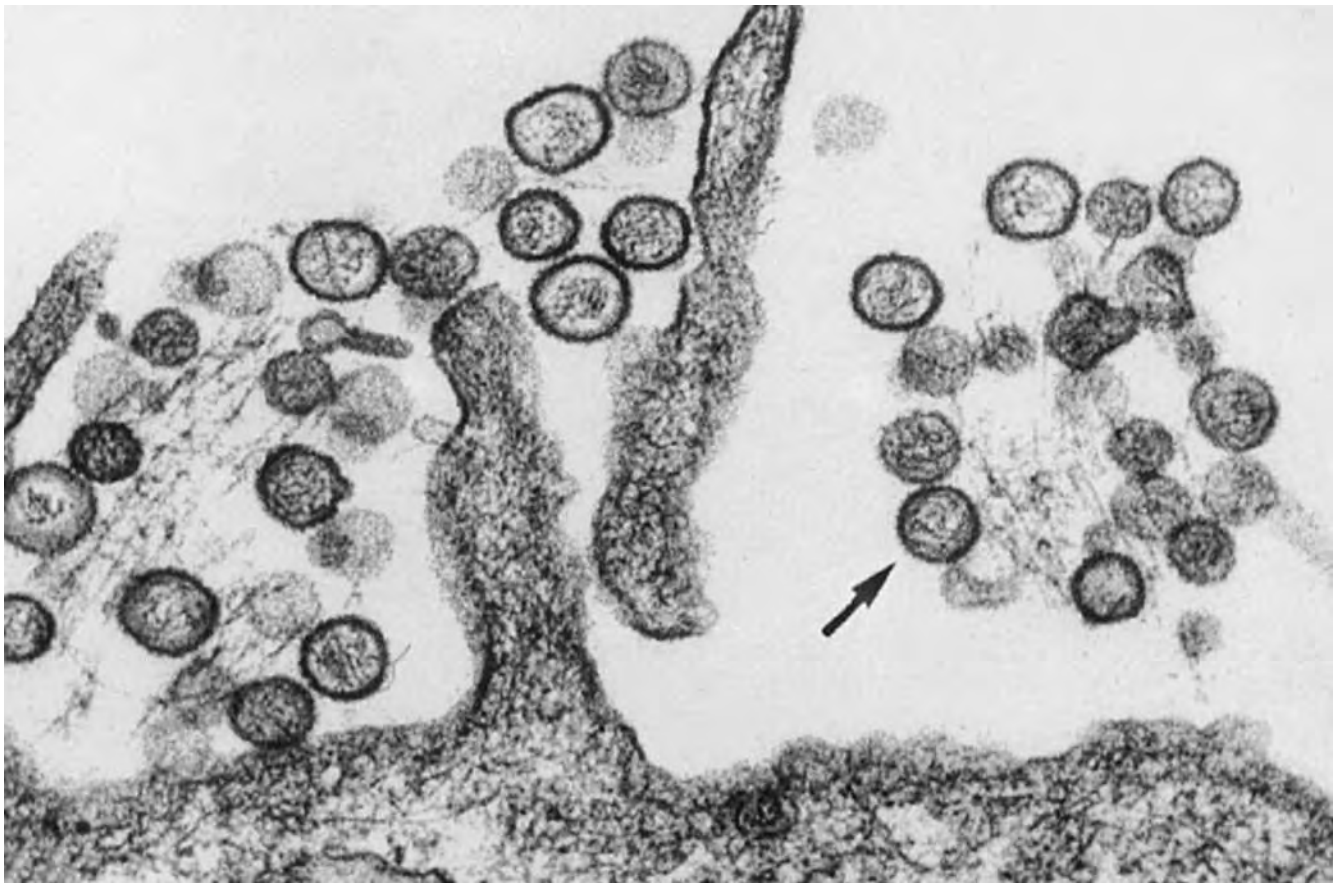
VIROLOGY, VIRAL CLASSIFICATION, TYPES OF VIRUSES

Virology is the discipline of microbiology that is concerned with the study of **viruses**. Viruses are essentially nonliving repositories of nucleic acid that require the presence of a living prokaryotic or eukaryotic cell for the replication of the nucleic acid.

Scientists who make virology their field of study are known as virologists. Not all virologists study the same things, as viruses can exist in a variety of hosts. Viruses can infect animals (including humans), plants, **fungi**, birds, aquatic organisms, **protozoa**, **bacteria**, and insects. Some viruses are able to infect several of these hosts, while other viruses are exclusive to one host.

All viruses share the need for a host in order to replicate their **deoxyribonucleic acid** (**DNA**) or **ribonucleic acid** (**RNA**). The virus commandeers the host's existing molecules for the nucleic acid replication process. There are a number of different viruses. The differences include the disease symptoms they cause, their antigenic composition, type of nucleic acid residing in the virus particle, the way the nucleic acid is arranged, the shape of the virus, and the fate of the replicated DNA. These differences are used to classify the viruses and have often been the basis on which the various types of viruses were named.

The classification of viruses operates by use of the same structure that governs the classification of bacteria. The International Committee on Taxonomy of Viruses established the viral classification scheme in 1966. From the broadest to the narrowest level of classification, the viral scheme is: Order, Family, Subfamily, Genus, Species, and Strain/type. To use an example, the virus that was responsible for an outbreak of Ebola hemorrhagic fever in a region of Africa called Kikwit is classified as Order Mononegavirales, Family *Filoviridae*, Genus *Filovirus*, and Species **Ebola virus** Zaire.



Thin section electron micrograph of adenoviruses.

In the viral classification scheme, all families end in the suffix **viridae**, for example Picornaviridae. Genera have the suffix **virus**. For example, in the family Picornaviridae there are five genera: enterovirus, cardiovirus, rhinovirus, apthovirus, and hepatovirus. The names of the genera typically derive from the preferred location of the virus in the body (for those viral genera that infect humans). As examples, rhinovirus is localized in the nasal and throat passages, and hepatovirus is localized in the liver. Finally, within each genera there can be several species.

As noted above, there are a number of criteria by which members of one grouping of viruses can be distinguished from those in another group. For the purposes of classification, however, three criteria are paramount. These criteria are the host organism or organisms that the virus utilizes, the shape of the virus particle, and the type and arrangement of the viral nucleic acid.

An important means of classifying viruses concerns the type and arrangement of nucleic acid in the virus particle. Some viruses have two strands of DNA, analogous to the double helix of DNA that is present in prokaryotes such as bacteria and in eukaryotic cells. Some viruses, such as the **Adenoviruses**, replicate in the **nucleus** of the host using the replication machinery of the host. Other viruses, such as the poxviruses, do not integrate in the host genome, but replicate in the **cytoplasm** of the host. Another example of a double-stranded DNA virus are the Herpesviruses.

Other viruses only have a single strand of DNA. An example is the Parvoviruses. Viruses such as the Parvoviruses replicate their DNA in the host's nucleus. The replication involves the formation of what is termed a negative-sense strand of DNA, which is a blueprint for the subsequent formation of the RNA and DNA used to manufacture the new virus particles.

The genome of other viruses, such as Reoviruses and Birnaviruses, is comprised of double-stranded RNA. Portions of the RNA function independently in the production of a number of so-called messenger RNAs, each of which produces a protein that is used in the production of new viruses.

Still other viruses contain a single strand of RNA. In some of the single-stranded RNA viruses, such as Picornaviruses, Togaviruses, and the **Hepatitis** A virus, the RNA is read in a direction that is termed "+ sense." The sense strand is used to make the protein products that form the new virus particles. Other single-stranded RNA viruses contain what is termed a negative-sense strand. Examples are the Orthomyxoviruses and the Rhabdoviruses. The negative strand is the blueprint for the formation of the messenger RNAs that are required for production of the various viral proteins.

Still another group of viruses have + sense RNA that is used to make a DNA intermediate. The intermediate is used to

manufacture the RNA that is eventually packaged into the new virus particles. The main example is the **Retroviruses** (the Human **Immunodeficiency** Viruses belong here). Finally, a group of viruses consist of double-stranded DNA that is used to produce a RNA intermediate. An example is the **Hepadnaviruses**.

An aspect of virology is the identification of viruses. Often, the diagnosis of a viral illness relies, at least initially, on the visual detection of the virus. For this analysis, samples are prepared for electron microscopy using a technique called negative staining, which highlights surface detail of the virus particles. For this analysis, the shape of the virus is an important feature.

A particular virus will have a particular shape. For example, viruses that specifically infect bacteria, the so-called bacteriophages, look similar to the Apollo lunar landing spacecraft. A head region containing the nucleic acid is supported on a number of spider-like legs. Upon encountering a suitable bacterial surface, the virus acts like a syringe, to introduce the nucleic acid into the cytoplasm of the bacterium.

Other viruses have different shapes. These include spheres, ovals, worm-like forms, and even irregular (pleomorphic) arrangements. Some viruses, such as the **influenza** virus, have projections sticking out from the surface of the virus. These are crucial to the infectious process.

As new species of eukaryotic and prokaryotic organisms are discovered, no doubt the list of viral species will continue to grow.

See also Viral genetics; Virus replication

VIRULENCE • *see* MICROBIOLOGY, CLINICAL

VIRUS REPLICATION

Viral replication refers to the means by which virus particles make new copies of themselves.

Viruses cannot replicate by themselves. They require the participation of the replication equipment of the host cell that they infect in order to replicate. The molecular means by which this replication takes place varies, depending upon the type of virus.

Viral replication can be divided up into three phases: initiation, replication, and release.

The initiation phase occurs when the virus particle attaches to the surface of the host cell, penetrates into the cell and undergoes a process known as uncoating, where the viral genetic material is released from the virus into the host cell's **cytoplasm**. The attachment typically involves the recognition of some host surface molecules by a corresponding molecule on the surface of the virus. These two molecules can associate tightly with one another, binding the virus particle to the surface. A well-studied example is the haemagglutinin receptor of the influenzae virus. The receptors of many other viruses have also been characterized.

A virus particle may have more than one receptor molecule, to permit the recognition of different host molecules, or of different regions of a single host molecule. The molecules on the host surface that are recognized tend to be those that are known as glycoproteins. For example, the **human immunodeficiency virus** recognizes a host glycoprotein called CD4. Cells lacking CD4 cannot, for example, bind the **HIV** particle.

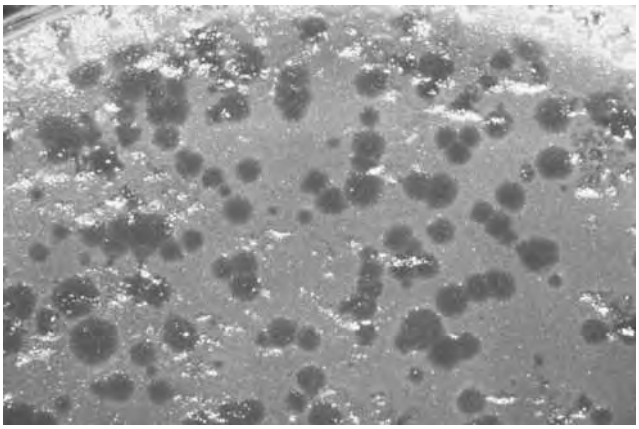
Penetration of the bound virus into the host interior requires energy. Accordingly, penetration is an active step, not a passive process. The penetration process can occur by several means. For some viruses, the entire particle is engulfed by a membrane-enclosed bag produced by the host (a vesicle) and is drawn into the cell. This process is called endocytosis. Polio virus and orthomyxovirus enters a cell via this route. A second method of penetration involves the fusion of the viral membrane with the host membrane. Then the viral contents are directly released into the host. HIV, paramyxoviruses, and **herpes** viruses use this route. Finally, but more rarely, a virus particle can be transported across the host membrane. For example, poliovirus can cause the formation of a pore through the host membrane. The viral **DNA** is then released into the pore and passes across to the inside of the host cell.

Once inside the host, the viruses that have entered via endocytosis or transport across the host membrane need to release their genetic material. With poxvirus, viral proteins made after the entry of the virus into the host are needed for uncoating. Other viruses, such as **adenoviruses**, herpesviruses, and papovaviruses associate with the host membrane that surrounds the **nucleus** prior to uncoating. They are guided to the nuclear membrane by the presence of so-called nuclear localization signals, which are highly charged viral proteins. The viral genetic material then enters the nucleus via pores in the membrane. The precise molecular details of this process remains unclear for many viruses.

For animal viruses, the uncoating phase is also referred to as the eclipse phase. No infectious virus particles can be detected during that 10–12 hour period of time.

In the replication, or synthetic, phase the viral genetic material is converted to **deoxyribonucleic acid** (DNA), if the material originally present in the viral particle is **ribonucleic acid** (**RNA**). This so-called reverse **transcription** process needs to occur in **retroviruses**, such as HIV. The DNA is imported into the host nucleus where the production of new DNA, RNA, and protein can occur. The replication phase varies greatly from virus type to virus type. However, in general, proteins are manufactured to ensure that the cell's replication machinery is harnessed to permit replication of the viral genetic material, to ensure that this replication of the genetic material does indeed occur, and to ensure that this newly made material is properly packaged into new virus particles.

Replication of the viral material can be a complicated process, with different stretches of the genetic material being transcribed simultaneously, with some of these **gene** products required for the transcription of other viral genes. Also replication can occur along a straight stretch of DNA, or when the DNA is circular (the so-called "rolling circle" form). RNA-containing viruses must also undergo a reverse transcription



Growth of virus causes clearing (plaques) in lawn of *Escherichia coli* culture on agar.

from DNA to RNA prior to packaging of the genetic material into the new virus particles.

In the final stage, the viral particles are assembled and exit the host cell. The assembly process can involve helper proteins, made by the virus or the host. These are also called **chaperones**. Other viruses, such as **tobacco mosaic virus**, do not need these helper chaperones, as the proteins that form the building blocks of the new particles spontaneously self-assemble. In most cases, the assembly of viruses is symmetrical; that is, the structure is the same throughout the viral particle. For example, in the tobacco mosaic virus, the proteins constituents associate with each other at a slight angle, producing a symmetrical helix. Addition of more particles causes the helix to coil "upward" forming a particle. An exception to the symmetrical assembly is the **bacteriophage**. These viruses have a head region that is supported by legs that are very different in structure. Bacteriophage assembly is very highly coordinated, involving the separate manufacture of the component parts and the direct fitting together of the components in a sequential fashion.

Release of viruses can occur by a process called budding. A membrane "bleb" containing the virus particle is formed at the surface of the cell and is pinched off. For herpes virus this is in fact how the viral membrane is acquired. In other words, the viral membrane is a host-derived membrane. Other viruses, such as bacteriophage, may burst the host cell, spewing out the many progeny virus particles. But many viruses do not adopt such a host destructive process, as it limits the time of an infection due to destruction of the host cells needed for future replication.

See also Herpes and herpes virus; Human immunodeficiency virus (HIV); Invasiveness and intracellular infection

VIRUSES AND RESPONSES TO VIRAL INFECTION

There are a number of different viruses that challenge the human **immune system** and that may produce disease in humans. In common, viruses are small, infectious agents that consist of a core of genetic material—either **deoxyribonucleic acid** (**DNA**) or **ribonucleic acid** (RNA)—surrounded by a shell of protein. Although precise mechanisms vary, viruses cause disease by infecting a host cell and commandeering the host cell's synthetic capabilities to produce more viruses. The newly made viruses then leave the host cell, sometimes killing it in the process, and proceed to infect other cells within the host. Because viruses invade cells, drug therapies have not yet been designed to kill viruses, although some have been developed to inhibit their growth. The human immune system is the main defense against a viral disease.

Bacterial viruses, called bacteriophages, infect a variety of **bacteria**, such as *Escherichia coli,* a bacteria commonly found in the human digestive tract. Animal viruses cause a variety of fatal diseases. **Acquired Immunodeficiency Syndrome** (**AIDS**) is caused by the **Human Immunodeficiency Virus** (**HIV**); **hepatitis** and **rabies** are viral diseases; and **hemorrhagic fevers**, which are characterized by severe internal bleeding, are caused by filoviruses. Other animal viruses cause some of the most common human diseases. Often these diseases strike in childhood. **Measles**, **mumps**, and chickenpox are viral diseases. The common **cold** and **influenza** are also caused by viruses. Finally, some viruses can cause cancer and tumors. One such virus, **Human T-cell Leukemia Virus** (**HTLV**), was only recently discovered and its role in the development of a particular kind of leukemia is still being elucidated.

Although viral structure varies considerably between the different **types of viruses**, all viruses share some common characteristics. All viruses contain either **RNA** or DNA surrounded by a protective protein shell called a capsid. Some viruses have a double strand of DNA, others a single strand of DNA. Other viruses have a double strand of RNA or a single strand of RNA. The size of the genetic material of viruses is often quite small. Compared to the 100,000 genes that exist within human DNA, viral genes number from 10 to about 200 genes.

Viruses contain such small amounts of genetic material because the only activity that they perform independently of a host cell is the synthesis of the protein capsid. In order to reproduce, a virus must infect a host cell and take over the host cell's synthetic machinery. This aspect of viruses—that the virus does not appear to be "alive" until it infects a host cell—has led to controversy in describing the nature of viruses. Are they living or non-living? When viruses are not inside a host cell, they do not appear to carry out many of the functions ascribed to living things, such as reproduction, **metabolism**, and movement. When they infect a host cell, they acquire these capabilities. Thus, viruses are both living and non-living. It was once acceptable to describe viruses as agents that exist on the boundary between living and non-living; however, a more accurate description of viruses is that they are either active or inactive, a description that leaves the question of life behind altogether.

All viruses consist of genetic material surrounded by a capsid; but variations exist within this basic structure. Studding the envelope of these viruses are protein "spikes." These spikes are clearly visible on some viruses, such as the

influenza viruses; on other enveloped viruses, the spikes are extremely difficult to see. The spikes help the virus invade host cells. The influenza virus, for instance, has two types of spikes. One type, composed of **hemagglutinin** protein (HA), fuses with the host cell membrane, allowing the virus particle to enter the cell. The other type of spike, composed of the protein neuraminidase (NA), helps the newly formed virus particles to bud out from the host cell membrane.

The capsid of viruses is relatively simple in structure, owing to the few genes that the virus contains to encode the capsid. Most viral capsids consist of a few repeating protein subunits. The capsid serves two functions: it protects the viral genetic material and it helps the virus introduce itself into the host cell. Many viruses are extremely specific, targeting only certain cells within the plant or animal body. HIV, for instance, targets a specific immune cell, the T helper cell. The cold virus targets respiratory cells, leaving the other cells in the body alone. How does a virus "know" which cells to target? The viral capsid has special receptors that match receptors on their targeted host cells. When the virus encounters the correct receptors on a host cell, it "docks" with this host cell and begins the process of infection and replication.

Most viruses are rod-shaped or roughly sphere-shaped. Rod-shaped viruses include **tobacco mosaic virus** and the filoviruses. Although they look like rods under a **microscope**, these viral capsids are actually composed of protein molecules arranged in a helix. Other viruses are shaped somewhat like spheres, although many viruses are not actual spheres. The capsid of the adenovirus, which infects the respiratory tract of animals, consists of 20 triangular faces. This shape is called an icosahedron. HIV is a true sphere, as is the influenza virus.

Some viruses are neither rod- nor sphere-shaped. The poxviruses are rectangular, looking somewhat like bricks. Parapoxviruses are ovoid. Bacteriophages are the most unusually shaped of all viruses. A **bacteriophage** consists of a head region attached to a sheath. Protruding from the sheath are tail fibers that dock with the host bacterium. Bacteriophage structure is eminently suited to the way it infects cells. Instead of the entire virus entering the bacterium, the bacteriophage injects its genetic material into the cell, leaving an empty capsid on the surface of the bacterium.

Viruses are obligate intracellular **parasites**, meaning that in order to replicate, they need to be inside a host cell. Viruses lack the machinery and **enzymes** necessary to reproduce; the only synthetic activity they perform on their own is to synthesize their capsids.

The infection cycle of most viruses follows a basic pattern. Bacteriophages are unusual in that they can infect a bacterium in two ways (although other viruses may replicate in these two ways as well). In the lytic cycle of replication, the bacteriophage destroys the bacterium it infects. In the lysogenic cycle, however, the bacteriophage coexists with its bacterial host and remains inside the bacterium throughout its life, reproducing only when the bacterium itself reproduces.

An example of a bacteriophage that undergoes lytic replication inside a bacterial host is the T4 bacteriophage, which infects *E. coli*. T4 begins the infection cycle by docking with an *E. coli* bacterium. The tail fibers of the bacteriophage make contact with the cell wall of the bacterium, and the bacteriophage then injects its genetic material into the bacterium. Inside the bacterium, the viral genes are transcribed. One of the first products produced from the viral genes is an enzyme that destroys the bacterium's own genetic material. Now the virus can proceed in its replication unhampered by the bacterial genes. Parts of new bacteriophages are produced and assembled. The bacterium then bursts, and the new bacteriophages are freed to infect other bacteria. This entire process takes only 20–30 minutes.

In the lysogenic cycle, the bacteriophage reproduces its genetic material but does not destroy the host's genetic material. The bacteriophage called lambda, another *E. coli*-infecting virus, is an example of a bacteriophage that undergoes lysogenic replication within a bacterial host. After the viral DNA has been injected into the bacterial host, it assumes a circular shape. At this point the replication cycle can become either lytic or lysogenic. In a lysogenic cycle the circular DNA attaches to the host cell genome at a specific place. This combination host-viral genome is called a prophage. Most of the viral genes within the prophage are repressed by a special repressor protein, so they do not encode the production of new bacteriophages. However, each time the bacterium divides, the viral genes are replicated along with the host genes. The bacterial progeny are thus lysogenically infected with viral genes.

Interestingly, bacteria that contain prophages can be destroyed when the viral DNA is suddenly triggered to undergo lytic replication. Radiation and chemicals are often the triggers that initiate lytic replication. Another interesting aspect of prophages is the role they play in human diseases. The bacteria that cause **diphtheria** and **botulism** both harbor viruses. The viral genes encode powerful toxins that have devastating effects on the human body. Without the infecting viruses, these bacteria may well be innocuous. It is the presence of viruses that makes these bacterial diseases so lethal.

Scientists have classified viruses according to the type of genetic material they contain. Broad categories of viruses include double-stranded DNA viruses, single-stranded DNA viruses, double-stranded RNA viruses, and single stranded RNA viruses. For the description of virus types that follows, however, these categories are not used. Rather, viruses are described by the type of disease they cause.

Poxviruses are the most complex kind of viruses known. They have large amounts of genetic material and fibrils anchored to the outside of the viral capsid that assist in attachment to the host cell. Poxviruses contain a double strand of DNA.

Viruses cause a variety of human diseases, including **smallpox** and **cowpox**. Because of worldwide **vaccination** efforts, smallpox has virtually disappeared from the world, with the last known case appearing in Somalia in 1977. The only places on Earth where smallpox virus currently exists are two labs: the **Centers for Disease Control** in Atlanta and the Research Institute for Viral Preparation in Moscow. Prior to the eradication efforts begun by the **World Health Organization** in 1966, smallpox was one of the most devastating of human diseases. In 1707, for instance, an outbreak of smallpox killed 18,000 of Iceland's 50,000 residents. In Boston in 1721,

smallpox struck 5,889 of the city's 12,000 inhabitants, killing 15% of those infected.

Edward Jenner (1749–1823) is credited with developing the first successful **vaccine** against a viral disease, and that disease was smallpox. A vaccine works by eliciting an immune response. During this immune response, specific immune cells, called memory cells, are produced that remain in the body long after the foreign microbe present in a vaccine has been destroyed. When the body again encounters the same kind of microbe, the memory cells quickly destroy the microbe. Vaccines contain either a live, altered version of a virus or bacteria, or they contain only parts of a virus or bacteria, enough to elicit an immune response.

In 1797, Jenner developed his smallpox vaccine by taking infected material from a cowpox lesion on the hand of a milkmaid. Cowpox was a common disease of the era, transmitted through contact with an infected cow. Unlike smallpox, however, cowpox is a much milder disease. Using the cowpox pus, he inoculated an eight-year-old boy. Jenner continued his vaccination efforts through his lifetime. Until 1976, children were vaccinated with the smallpox vaccine, called vaccinia. Reactions to the introduction of the vaccine ranged from a mild fever to severe complications, including (although very rarely) death. In 1976, with the eradication of smallpox complete, vaccinia vaccinations for children were discontinued, although vaccinia continues to be used as a carrier for recombinant DNA techniques. In these techniques, foreign DNA is inserted in cells. Efforts to produce a vaccine for HIV, for instance, have used vaccinia as the vehicle that carries specific parts of HIV.

Herpesviruses are enveloped, double-stranded DNA viruses. Of the more than 50 **herpes** viruses that exist, only eight cause disease in humans. These include the human herpes virus types 1 and 2 that cause cold sores and genital herpes; human herpes virus 3, or varicella-zoster virus (VZV), that causes chickenpox and shingles; cytomegalovirus (CMV), a virus that in some individuals attacks the cells of the eye and leads to blindness; human herpes virus 4, or **Epstein-Barr virus** (EBV), which has been implicated in a cancer called Burkitt's lymphoma; and human herpes virus types 6 and 7, newly discovered viruses that infect white blood cells. In addition, herpes B virus is a virus that infects monkeys and can be transmitted to humans by handling infected monkeys.

Adenoviruses are viruses that attack respiratory, intestinal, and eye cells in animals. More than 40 kinds of human adenoviruses have been identified. Adenoviruses contain double-stranded DNA within a 20-faceted capsid. Adenoviruses that target respiratory cells cause bronchitis, **pneumonia**, and tonsillitis. Gastrointestinal illnesses caused by adenoviruses are usually characterized by diarrhea and are often accompanied by respiratory symptoms. Some forms of appendicitis are also caused by adenoviruses. Eye illnesses caused by adenoviruses include conjunctivitis, an infection of the eye tissues, as well as a disease called pharyngoconjunctival fever, a disease in which the virus is transmitted in poorly chlorinated swimming pools.

Human papoviruses include two groups: the papilloma viruses and the polyomaviruses. Human papilloma viruses (HPV) are the smallest double-stranded DNA viruses. They replicate within cells through both the lytic and the lysogenic replication cycles. Because of their lysogenic capabilities, HPV-containing cells can be produced through the replication of those cells that HPV initially infects. In this way, HPV infects epithelial cells, such as the cells of the skin. HPVs cause several kinds of benign (non-cancerous) warts, including plantar warts (those that form on the soles of the feet) and genital warts. However, HPVs have also been implicated in a form of cervical cancer that accounts for 7% of all female cancers.

HPV is believed to contain oncogenes, or genes that encode for growth factors that initiate the uncontrolled growth of cells. This uncontrolled proliferation of cells is called cancer. When the HPV oncogenes within an epithelial cell are activated, they cause the epithelial cell to proliferate. In the cervix (the opening of the uterus), the cell proliferation manifests first as a condition called cervical neoplasia. In this condition, the cervical cells proliferate and begin to crowd together. Eventually, cervical neoplasia can lead to full-blown cancer.

Polyomaviruses are somewhat mysterious viruses. Studies of blood have revealed that 80% of children aged five to none years have antibodies to these viruses, indicating that they have at some point been exposed to polyomaviruses. However, it is not clear what disease this virus causes. Some evidence exists that a mild respiratory illness is present when the first antibodies to the virus are evident. The only disease that is certainly caused by polyomavirses is called progressive multifocal leukoencephalopathy (PML), a disease in which the virus infects specific brain cells called the oligodendrocytes. PML is a debilitating disease that is usually fatal, and is marked by progressive neurological degeneration. It usually occurs in people with suppressed immune systems, such as cancer patients and people with AIDS.

The **hepadnaviruses** cause several diseases, including hepatitis B. Hepatitis B is a chronic, debilitating disease of the liver and immune system. The disease is much more serious than hepatitis A for several reasons: it is chronic and long-lasting; it can cause cirrhosis and cancer of the liver; and many people who contract the disease become carriers of the virus, able to transmit the virus through body fluids such as blood, semen, and vaginal secretions.

The hepatitis B virus (HBV) infects liver cells and has one of the smallest viral genomes. A double-stranded DNA virus, HBV is able to integrate its genome into the host cell's genome. When this integration occurs, the viral genome is replicated each time the cell divides. Individuals who have integrated HBV into their cells become carriers of the disease. Recently, a vaccine against HBV was developed. The vaccine is especially recommended for health care workers who through exposure to patient's body fluids are at high risk for infection.

Parvoviruses are icosahedral, single-stranded DNA viruses that infect a wide variety of mammals. Each type of parvovirus has its own host. For instance, one type of parvovirus causes disease in humans; another type causes disease in cats; while still another type causes disease in dogs. The disease caused by parvovirus in humans is called erythremia infectiosum, a disease of the red blood cells that is

relatively rare except for individuals who have the inherited disorder sickle cell anemia. Canine and feline parvovirus infections are fatal, but a vaccine against parvovirus is available for dogs and cats.

Orthomyxoviruses cause influenza ("flu"). This highly contagious viral infection can quickly assume epidemic proportions, given the right environmental conditions. An influenza outbreak is considered an epidemic when more than 10% of the population is infected. Antibodies that are made against one type of rhinovirus are often ineffective against other types of viruses. For this reason, most people are susceptible to colds from season to season.

These helical, enveloped, single-stranded RNA viruses cause pneumonia, croup, measles, and mumps in children. A vaccine against measles and mumps has greatly reduced the incidence of these diseases in the United States. In addition, a paramyxovirus called respiratory syncytial virus (RSV) causes bronchiolitis (an infection of the bronchioles) and pneumonia.

Flaviviruses (from the Latin word meaning "yellow") cause insect-carried diseases including **yellow fever**, an often-fatal disease characterized by high fever and internal bleeding. Flaviviruses are single-stranded RNA viruses.

The two filoviruses, **Ebola virus** and Marburg virus, are among the most lethal of all human viruses. Both cause severe fevers accompanied by internal bleeding, which eventually kills the victim. The fatality rate of Marburg is about 60%, while the fatality rate of Ebola virus approaches 90%. Both are transmitted through contact with body fluids. Marburg and Ebola also infect primates.

Rhabdoviruses are bullet-shaped, single-stranded RNA viruses. They are responsible for rabies, a fatal disease that affects dogs, rodents, and humans.

Retroviruses are unique viruses. They are double-stranded RNA viruses that contain an enzyme called reverse transcriptase. Within the host cell, the virus uses reverse transcriptase to make a DNA copy from its RNA genome. In all other organisms, RNA is synthesized from DNA. Cells infected with retroviruses are the only living things that reverse this process.

The first retroviruses discovered were viruses that infect chickens. The Rous sarcoma virus, discovered in the 1950s by **Peyton Rous** (1879–1970), was also the first virus that was linked to cancer. However, it was not until 1980 that the first human retrovirus was discovered. Called Human T-cell Leukemia Virus (HTLV), this virus causes a form of leukemia called adult T-cell leukemia. In 1983, another human retrovirus, Human **Immunodeficiency** Virus, the virus responsible for AIDS, was discovered independently by two researchers. Both HIV and HTLV are transmitted in body fluids.

See also Bacteria and bacterial infection; Epidemics, viral; Immune stimulation, as a vaccine; Immunity, active, passive, and delayed; Immunology; Virology; Virus replication

VITAL STAINS

• *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

VOZROZHDENIYE ISLAND

Vozrozhdeniye island is located in the Aral Sea approximately 1,300 miles (2,092 km) to the east of Moscow. The island was used as biological weapons test site for the former Soviet Union. Now decommissioned, the island has served for decades as the repository of a large quantity of spores of *Bacillus anthracis*, the bacterial agent of **anthrax**, and other disease-causing **bacteria** and **viruses**.

Vozrozhdeniye island translates as Renaissance island. The island was used for open-air testing of bioweapons. The sparse vegetation on the island, remote location, and summer temperatures that reach 140°F (60°C) reduced the chances that escaping bioweapons would survive. Besides the testing of anthrax bioweapons, Soviet archives indicate that the microbial agents of **tularemia**, plague, typhoid, and possibly **smallpox** were used for experimentation.

The **biological warfare** agents buried on the island were supposed to have been destroyed following the signing of a treaty with the Soviet Union banning the manufacture and use of such weapons. Similar weapons manufactured for the same reason by the United States were reportedly destroyed in 1972. The bioweapons were manufactured by Soviet Union as part of their Cold War–inspired biological warfare program. They were buried on the island in 1988. The island has been abandoned since 1991 by the Russian government.

Vozrozhdeniye island has remained unguarded since that time. The main reason has been the isolated location of the facility in the middle of the Aral Sea. Over the past two decades, irrigation demands for water have depleted the freshwater sea to such an extent that the sea is becoming smaller. Many scientists now fear that Vozrozhdeniye Island might soon be directly connected to the mainland, making the stockpiled weapons more vulnerable to bioterrorist theft.

Additionally, indications are that some of the buried bioweapons are migrating towards the surface. Once exposed, some of the materials could be aerosolized and spread by the wind, or transported by birds.

The anthrax buried on the island was designed especially for the lethal use on humans in the time of war. The powder is a freeze-dried form of the bacteria called a spore. The spore is a dormant form of the bacterium that allows the persistence of the genetic material for very long periods of time. Resuscitation of the spore requires only suspension in growth media having the appropriate nutrients and incubation of the suspension at a temperature that is hospitable for the **bacterial growth**. Direct inhalation of the spores produces a lethal form of anthrax.

See also Bioterrorism, protective measures; Containment and release prevention protocol

WAKSMAN, SELMAN ABRAHAM (1888-1973)

Russian-born American microbiologist

Selman Waksman discovered life-saving antibacterial compounds and his investigations spawned further studies for other disease-curing drugs. Waksman isolated streptomycin, the first chemical agent effective against **tuberculosis**. Prior to Waksman's discovery, tuberculosis was often a lifelong debilitating disease, and was fatal in some forms. Streptomycin effected a powerful and wide-ranging cure, and for this discovery, Waksman received the 1952 Nobel Prize in physiology or medicine. In pioneering the field of antibiotic research, Waksman had an inestimable impact on human health.

The only son of a Jewish furniture textile weaver, Selman Abraham Waksman was born in the tiny Russian village of Novaya Priluka on July 22, 1888. Life was hard in late-nineteenth-century Russia. Waksman's only sister died from **diphtheria** when he was nine. There were particular tribulations for members of a persecuted ethnic minority. As a teen during the Russian revolution, Waksman helped organize an armed Jewish youth defense group to counteract oppression. He also set up a school for underprivileged children and formed a group to care for the sick. These activities prefaced his later role as a standard-bearer for social responsibility.

Several factors led to Waksman's immigration to the United States. He had received his diploma from the *Gymnasium* in Odessa and was poised to attend university, but he doubtless recognized the very limited options he held as a Jew in Russia. At the same time, in 1910, his mother died, and cousins who had immigrated to New Jersey urged him to follow their lead. Waksman did so, and his move to a farm there, where he learned the basics of scientific farming from his cousin, likely had a pivotal influence on Waksman's later choice of field of study.

In 1911 Waksman enrolled in nearby Rutgers College (later University) of Agriculture, following the advice of fellow Russian immigrant Jacob Lipman, who led the college's bacteriology department. He worked with Lipman, developing a fascination with the **bacteria** of soil, and graduated with a B.S. in 1915. The next year he earned his M.S. degree. Around this time, he also became a naturalized United States citizen and changed the spelling of his first name from Zolman to Selman. Waksman married Bertha Deborah Mitnik, a childhood sweetheart and the sister of one of his childhood friends, in 1916. Deborah Mitnik had come to the United States in 1913, and in 1919 she bore their only child, Byron Halsted Waksman, who eventually went on to a distinguished career at Yale University as a pathology professor.

Waksman's intellect and industry enabled him to earn his Ph.D. in less than two years at the University of California, Berkeley. His 1918 dissertation focused on proteolytic **enzymes** (special proteins that break down proteins) in **fungi**. Throughout his schooling, Waksman supported himself through various scholarships and jobs. Among the latter were ranch work, caretaker, night watchman, and tutor of English and science.

Waksman's former advisor invited him to join Rutgers as a lecturer in soil bacteriology in 1918. He was to stay at Rutgers for his entire professional career. When Waksman took up the post, however, he found his pay too low to support his family. Thus, in his early years at Rutgers he also worked at the nearby Takamine Laboratory, where he produced enzymes and ran toxicity tests.

In the 1920s Waksman's work gained recognition in scientific circles. Others sought out his keen mind, and his prolific output earned him a well-deserved reputation. He wrote two major books during this decade. *Enzymes: Properties, Distribution, Methods, and Applications,* coauthored with Wilburt C. Davison, was published in 1926, and in 1927 his thousand-page *Principles of Soil Microbiology* appeared. This latter volume became a classic among soil bacteriologists. His laboratory produced more than just books. One of Waksman's students during this period was **René Dubos**, who would later discover the antibiotic gramicidin, the first chemotherapeutic

Selman Waksman won the 1952 Nobel prize in Physiology or Medicine for his discovery of streptomycin, the first antibiotic effective against the bacterium that causes tuberculosis.

agent effective against gram-positive bacteria (bacteria that hold dye in a stain test named for Danish bacteriologist Hans Gram). Waksman became an associate professor at Rutgers in the mid–1920s and advanced to the rank of full professor in 1930.

During the 1930s Waksman systematically investigated the complex web of microbial life in soil, humus, and peat. He was recognized as a leader in the field of soil microbiology, and his work stimulated an ever-growing group of graduate students and postdoctoral assistants. He continued to publish widely, and he established many professional relationships with industrial firms that utilized products of microbes. These companies that produced enzymes, pharmaceuticals, vitamins, and other products were later to prove valuable in Waksman's researches, mass-producing and distributing the products he developed. Among his other accomplishments during this period was the founding of the division of Marine Bacteriology at Woods Hole Oceanographic Institution in 1931. For the next decade he spent summers there and eventually became a trustee, a post he filled until his death.

In 1939, Waksman was appointed chair of the U.S. War Committee on Bacteriology. He derived practical applications from his earlier studies on soil **microorganisms**, developing antifungal agents to protect soldiers and their equipment. He also worked with the Navy on the problem of bacteria that attacked ship hulls. Early that same year Dubos announced his finding of two antibacterial substances, tyrocidine, and gramicidin, derived from a soil bacterium (*Bacillus brevis*). The latter compound, effective against gram-positive bacteria, proved too toxic for human use but did find widespread employment against various bacterial infections in veterinary medicine. The discovery of gramicidin also evidently inspired Waksman to dedicate himself to focus on the medicinal uses of antibacterial soil microbes. It was in this period that he began rigorously investigating the antibiotic properties of a wide range of soil fungi.

Waksman set up a team of about 50 graduate students and assistants to undertake a systematic study of thousands of different soil fungi and other microorganisms. The rediscovery at this time of the power of **penicillin** against gram-positive bacteria likely provided further incentive to Waksman to find an antibiotic effective against gram-negative bacteria, which include the kind that causes tuberculosis.

In 1940, Waksman became head of Rutgers' department of microbiology. In that year too, with the help of Boyd Woodruff, he isolated the antibiotic actinomycin. Named for the actinomycetes (rod- or filament-shaped bacteria) from which it was isolated, this compound also proved too toxic for human use, but its discovery led to the subsequent finding of variant forms (actinomycin A, B, C, and D), several of which were found to have potent anti-cancer effects. Over the next decade Waksman isolated 10 distinct **antibiotics**. It is Waksman who first applied the term antibiotic, which literally means against life, to such drugs.

Among these discoveries, Waksman's finding of streptomycin had the largest and most immediate impact. Not only did streptomycin appear nontoxic to humans, however, it was highly effective against gram-negative bacteria. (Prior to this time, the antibiotics available for human use had been active only against the gram-positive strains.) The importance of streptomycin was soon realized. Clinical trials showed it to be effective against a wide range of diseases, most notably tuberculosis.

At the time of streptomycin's discovery, tuberculosis was the most resistant and irreversible of all the major infectious diseases. It could only be treated with a regime of rest and nutritious diet. The tuberculosis bacillus consigned its victims to a lifetime of invalidism and, when it invaded organs other than the lungs, often killed. Sanatoriums around the country were filled with persons suffering the ravages of tuberculosis, and little could be done for them.

Streptomycin changed all of that. From the time of its first clinical trials in 1944, it proved to be remarkably effective against tuberculosis, literally snatching sufferers back from the jaws of death. By 1950, streptomycin was used against seventy different germs that were not treatable with penicillin. Among the diseases treated by streptomycin were bacterial **meningitis** (an **inflammation** of membranes enveloping the brain and spinal cord), endocarditis (an inflammation of the lining of the heart and its valves), pulmonary and urinary tract infections, **leprosy**, **typhoid fever**, bacillary **dysentery**, cholera, and **bubonic plague**.

Waksman arranged to have streptomycin produced by a number of pharmaceutical companies, since demand for it soon skyrocketed beyond the capacity of any single company. Manufacture of the drug became a $50-million-per-year industry. Thanks to Waksman and streptomycin, Rutgers received millions of dollars of income from the royalties. Waksman donated much of his own share to the establishment of an Institute of Microbiology there. He summarized his early researches on the drug in *Streptomycin: Nature and Practical Applications* (1949). Streptomycin ultimately proved to have some human toxicity and was supplanted by other antibiotics,

but its discovery changed the course of modern medicine. Not only did it directly save countless lives, but its development stimulated scientists around the globe to search the microbial world for other antibiotics and medicines.

In 1949, Waksman isolated neomycin, which proved effective against bacteria that had become resistant to streptomycin. Neomycin also found a broad niche as a topical antibiotic. Other antibiotics soon came forth from his Institute of Microbiology. These included streptocin, framicidin, erlichin, candidin, and others. Waksman himself discovered eighteen antibiotics during the course of his career.

Waksman served as director of the Institute for Microbiology until his retirement in 1958. Even after that time, he continued to supervise research there. He also lectured widely and continued to write at the frenetic pace established early in his career. He eventually published more than twenty-five books, among them the autobiography *My Life with the Microbes,* and hundreds of articles. He was author of popular pamphlets on the use of thermophilic (heat-loving) microorganisms in composting and on the enzymes involved in jelly-making. He wrote biographies of several noted microbiologists, including his own mentor, Jacob Lipman. These works are in addition to his numerous publications in the research literature.

On August 16, 1973, Waksman died suddenly in Hyannis, Massachusetts, of a cerebral hemorrhage. He was buried near the institute to which he had contributed so much over the years. Waksman's honors over his professional career were many and varied. In addition to the 1952 Nobel Prize, Waksman received the French Legion of Honor, a Lasker award for basic medical science, elected a fellow of the American Association for the Advancement of Science, and received numerous commendations from academies and scholarly societies around the world.

See also Antibiotic resistance, tests for; Bacteria and bacterial infection; Streptococci and streptococcal infections

VON WASSERMAN, AUGUST PAUL (1866-1925)

German bacteriologist

August Paul von Wasserman was a German physician and bacteriologist. He is most noteworthy in the **history of microbiology** for his invention of the first test for the sexually transmitted disease of **syphilis**. The test is known as the **Wasserman test**.

Wasserman was born in 1866 in Bamberg, Germany. His entire education was received in that country. Wasserman received his undergraduate bacteriology degree and medical training at the universities of Erlanger, Vienna, Munich, and Strasbourg. He graduated from Strasbourg in 1888. Beginning in 1890, Wasserman joined **Robert Koch** at the latter's Institute for Infectious Diseases in Berlin. He became head of the institute's Department of Therapeutics and Serum research in 1907. In 1913, Wasserman left the Koch institute and joined the faculty at the Kaiser Wilhelm Institute, where he served as the Director of Experimental Therapeutics until his death in 1925.

Wasserman is remembered for a number of bacteriological accomplishments. He devised a test for **tuberculosis** and developed an antitoxin that was active against **diphtheria**. But his most noteworthy accomplishment occurred while he was still at the Institute for Infectious Diseases. In 1906, he developed a test for the presence of *Treponema pallidum* in humans. The bacterium is a spirochaete and is the cause of syphilis. The test became known as the Wasserman test.

The basis of the test is the production of antibodies to the syphilis bacterium and the ability of those antibodies to combine with known antigens in a solution. The antibody-antigen combination prevents a component called **complement** from subsequently destroying red blood cells. Clearing of the test solution (e.g., destruction of the red blood cells) is diagnostic for the absence of antibodies to *Treponema pallidum*.

The Wasserman test represents the first so-called complement test. In the decades since its introduction the Wasserman's test for syphilis has been largely superseded by other methods. But, the test is still reliable enough to be performed even to the present day in the diagnosis of syphilis.

See also Complement; Sexually transmitted diseases

WASSERMAN TEST

The Wasserman test is used to diagnose the illness known as **syphilis**. The test is named after its developer, the German bacteriologist August Wasserman (1866–1925). The Wasserman test was devised in 1906.

The Wasserman test is used to detect the presence of the bacterium that causes syphilis, the spirochete (spiral-shaped microorganism) *Treponema pallidum*. The basis of the test is the reaction of the **immune system** to the presence of the bacterium. Specifically, the test determines the presence or absence of an **antibody** that is produced in response to the presence of a constituent of the membrane of *Treponema pallidum*. The particular constituent is the membrane phospholipid.

The Wasserman test represents one of the earliest applications of an immunological reaction that is termed **complement** fixation. In the test, a patient's serum is heated to destroy a molecule called complement. A known amount of complement (typically from a guinea pig) is then added to the patient's serum. Next, the **antigen** (the bacterial phospholipid) is added along with red blood cells from sheep. The natural action of complement is to bind to the red blood cells and cause them to lyse (burst). Visually, this is evident as a clearing of the red-colored suspension. However, if the added antigen has bound to antibody that is present in the suspension, the complement becomes associated with the antigen-antibody complex. In technical terms, the complement is described being "fixed." Thus, if lysis of the red blood cells does not occur, then antibody to *Treponema pallidum* is present in the patient's serum, and allows a positive diagnosis for syphilis.

The Wasserman test is still used in the diagnosis of syphilis. However, the test has been found to be limiting, as

antibodies to the bacterium are not prevalent in the early stages of the disease. Thus, a patient who had contracted syphilis—but who is in the earliest stages of infection—could produce a negative Wasserman test. This can compromise patient health and treatment, as syphilis becomes more serious as the disease progresses with time.

See also Bacteria and bacterial infection; Laboratory techniques in immunology

WASTEWATER TREATMENT

Wastewater includes the sewage-bearing water that is flushed down toilets as well as the water used to wash dishes and for bathing. Processing plants use water to wash raw material and in other stages of the wastewater treatment production process. The treatment of water that exits households, processing plants and other institutions is a standard, even mandated, practice in many countries around the world. The purpose of the treatment if to remove compounds and **microorganisms** that could pollute the water to which the wastewater is discharged. Particularly with respect to microorganisms, the sewage entering a treatment plant contains extremely high numbers of **bacteria**, **viruses**, and **protozoa** that can cause disease if present in drinking water. Wastewater treatment lowers the numbers of such disease-causing microbes to levels that are deemed to be acceptable from a health standpoint. As well, organic matter, solids, and other pollutants are removed.

Wastewater treatment is typically a multi-stage process. Typically, the first step is known as the preliminary treatment. This step removes or grinds up large material that would otherwise clog up the tanks and equipment further on in the treatment process. Large matter can be retained by screens or ground up by passage through a grinder. Examples of items that are removed at this stage are rags, sand, plastic objects, and sticks.

The next step is known as primary treatment. The wastewater is held for a period of time in a tank. Solids in the water settle out while grease, which does not mix with water, floats to the surface. Skimmers can pass along the top and bottom of the holding tank to remove the solids and the grease. The clarified water passes to the next treatment stage, which is known as secondary treatment.

During secondary treatment, the action of microorganisms comes into play. There are three versions of secondary treatment. One version, which was developed in the mid-nineteenth century, is called the fixed film system. The fixed film in such a system is a film of microorganisms that has developed on a support such as rocks, sand, or plastic. If the film is in the form of a sheet, the wastewater can be overlaid on the fixed film. The domestic septic system represents such a type of fixed film. Alternatively, the sheets can be positioned on a rotating arm, which can slowly sweep the microbial films through the tank of wastewater. The microorganisms are able to extract organic and inorganic material from the wastewater to use as nutrients for growth and reproduction. As the microbial film thickens and matures, the metabolic activity of the film increases. In this way, much of the organic and inorganic load in the wastewater can be removed.

Another version of secondary treatment is called the suspended film. Instead of being fixed on a support, microorganisms are suspended in the wastewater. As the microbes acquire nutrients and grow, they form aggregates that settle out. The settled material is referred to as sludge. The sludge can be scrapped up and removed. As well, some of the sludge is added back to the wastewater. This is analogous to inoculating growth media with microorganisms. The microbes in the sludge now have a source of nutrients to support more growth, which further depletes the wastewater of the organic waste. This cycle can be repeated a number of times on the same volume of water.

Sludge can be digested and the methane that has been formed by bacterial **fermentation** can be collected. Burning of the methane can be used to produce electricity. The sludge can also be dried and processed for use as compost.

A third version of secondary treatment utilizes a specially constructed lagoon. Wastewater is added to a lagoon and the sewage is naturally degraded over the course of a few months. The algae and bacteria in the lagoon consume nutrients such as phosphorus and nitrogen. Bacterial activity produces carbon dioxide. Algae can utilize this gas, and the resulting algal activity produces oxygen that fuels bacterial activity. A cycle of microbiological activity is established.

Bacteria and other microorganisms are removed from the wastewater during the last treatment step. Basically, the final treatment involves the addition of disinfectants, such as chlorine compounds or ozone, to the water, passage of the water past ultraviolet lamps, or passage of the water under pressure through membranes whose very small pore size impedes the passage of the microbes. In the case of ultraviolet irradiation, the wavelength of the lamplight is lethally disruptive to the genetic material of the microorganisms. In the case of disinfectants, neutralization of the high concentration of the chemical might be necessary prior to discharge of the treated water to a river, stream, lake, or other body of water. For example, chlorinated water can be treated with sulfur dioxide.

Chlorination remains the standard method for the final treatment of wastewater. However, the use of the other systems is becoming more popular. Ozone treatment is popular in Europe, and membrane-based or ultraviolet treatments are increasingly used as a supplement to chlorination.

Within the past several decades, the use of sequential treatments that rely on the presence of living material such as plants to treat wastewater by filtration or metabolic use of the pollutants has become more popular. These systems have been popularly dubbed "living machines." Restoration of wastewater to near drinking **water quality** is possible.

Wastewater treatment is usually subject to local and national standards of operational performance and quality in order to ensure that the treated water is of sufficient quality so as to pose no threat to aquatic life or settlements downstream that draw the water for drinking.

See also Biodegradable substances; Biofilm formation and dynamic behavior; Disinfection and disinfectants; Disposal of

A wastewater treatment plant in Detroit, Michigan.

infectious microorganisms; Economic uses and benefits of microorganisms; Growth and growth media; Public health, current issues; Radiation mutagenesis; Water pollution and purification; Water quality

WATER POLLUTION AND PURIFICATION

With respect to **microorganisms**, water pollution refers to the presence in water of microbes that originated from the intestinal tract of humans and other warm-blooded animals. Water pollution can also refer to the presence of compounds that promote the growth of the microbes. The remediation of polluted water—the removal of the potentially harmful microorganisms—or the reduction of their numbers to levels considered to be acceptable for whatever purpose the water is used, represents the purification of water.

Microorganisms that reside in the intestinal tract find their way into fresh and marine water when feces contaminate the water. Examples of **bacteria** that can pollute water in this way are *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio cholerae*. Warm-blooded animals other than humans can also contribute protozoan **parasites** to the water via their feces. The two prominent examples of health relevance to humans are *Cryptosporidium parvum* and *Giardia lamblia*. The latter two species are becoming more prominent. They are also resistant to chlorine, the most popular purification chemical.

Normally, the intestinal bacteria do not survive long in the inhospitable world of the water. But, if they are ingested while still living, they can cause maladies, ranging from inconvenient intestinal upset to life-threatening infections. A prominent example of the latter is *Escherichia coli O157:H7*. Pollution of the water with this strain can cause severe intestinal damage, life long damage to organs such as the kidney and—especially in the young, elderly and those whose immune systems are compromised—death.

There are several common ways in which microorganisms can pollute water. Runoff from agricultural establishments, particularly where livestock is raised, is one route of **contamination**. Seasonal runoff can occur, especially in the springtime when rainfall is more pronounced. The feeding of birds (e.g., ducks) is now recognized as a contributing factor. For example, a large numbers of ducks that congregate can contribute large quantities of fecal material to localized ponds and lakes.

Once in the water, the growth of microorganisms can be exacerbated by environmental factors such as the water temperature, and by the chemical composition of the water. For

Sampling polluted water.

example, runoff of fertilizers from suburban properties can infuse watercourses with nitrogen, potassium, and phosphorus. All these are desirable nutrients for **bacterial growth**.

Water purification seeks to convert the polluted water into water that is acceptable for drinking, for recreation, or for some other purpose. Techniques such as filtration and exposure to agents or chemicals that will kill the microorganisms in the water are common means of purification. The use of **chlorination** remains the most widely used purification option. Others approaches are the use of ultraviolet radiation, filters of extremely small pore size (such that even **viruses** are excluded), and the use of a chemical known as ozone. Depending on the situation and the intended use of the finished water, combinations of these techniques can be used.

Purification of drinking water aims to remove as many bacteria as possible, and to completely eliminate those bacteria of intestinal origin. Recreational waters need not be that pristine. But bacterial numbers need to be below whatever standard has been deemed permissible for the particular local.

Another microbiological aspect of water pollution that has become recognized only within the past several years has been the presence in water of agents used to treat bacteria in other environments. For example in the household a number of disinfectant compounds are routinely employed in the cleaning of household surfaces. In the hospital, the use of **antibiotics** to kill bacteria is an everyday occurrence. Such materials have been detected in water both before and after municipal **wastewater treatment**. The health effect of these compounds is not known at the present time. However, by analogy with other systems, the low concentration of such compounds might provide selective pressure for the development of resistant bacterial populations.

See also Chlorination; Waste water treatment; Water quality

WATER QUALITY

Water is the universal solvent. Many compounds that can dissolve in water are used as food sources by a variety of microbiological life forms. These **microorganisms** are themselves water-based and their constituent molecules are designed to function in aqueous environments. Thus, water can widely support the growth of microorganisms.

Some of this growth is advantageous. For example, the strains of **yeast** whose fermentative abilities make possible the brewing of beer, the production of wine, and the baking of bread. In addition, the growth of **bacteria** in polluted water is used as a means of decontaminating the water. The bacteria

Collecting water for analysis.

are able to use the pollutant compound as a food source. In contrast, some forms of microbial growth can detrimental to products being produced or dangerous to the health of people consuming the water. Ensuring the quality of water from a microbiological standpoint is thus of extreme importance.

The main concern surrounding water quality is the freedom of the water from microorganisms that can cause disease. Typically, these agents are associated with the intestinal tract of warm-blooded animals including humans. Examples of disease causing bacteria are those in the genera of *Salmonella*, *Shigella*, and *Vibrio*. As well certain types of the intestinal bacterium *Escherichia coli* can cause infections. *Escherichia coli* O157:H7 has become prominent in the past decade. **Contamination** of drinking water with O157:H7 can be devastating. An infamous example of this is the contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000. Several thousand people became ill, and seven people died as a direct result of the O157:H7 infection.

The contamination of the well water in Walkerton occurred because of run-off from adjacent cattle farms. This route of water contamination is common. For this reason, the surveillance of wells for the presence of bacteria is often done more frequently following a heavy rain, or at times of the year when precipitation is marked.

The intestinal tract also harbors **viruses** that can contaminate water and cause disease. Some examples of these viruses are rotavirus, enteroviruses, and coxsackievirus.

A number of protozoan microorganisms are also problematic with respect to water quality. The two most prominent protozoans are in the genera *Giardia* and *Cryptosporidium*. These microorganisms are resident in the intestinal tract of animals such as beaver and deer. Their increasing prevalence in North America is a consequence of the increasing encroachment of civilized areas on natural areas.

Municipal drinking water is usually treated in order to minimize the risk of the contamination of the water with the above microbes. Similarly, the protection of water quality by the boiling of the water has long been known. Even today, so-called "boil water orders" are issued in municipalities when the water quality is suspect. The addition of disinfectant compounds, particularly chlorine or derivatives of chlorine, is a common means utilized to kill bacteria in water. Other treatments that kill bacteria include the use of a gaseous ozone, and irradiation of water with ultraviolet light to disrupt bacterial genetic material. In more recent decades, the filtering of water has been improved so that now filters exist that can exclude even particles as tiny as viruses from the treated (or "finished") water. The killing of the protozoan microorganisms

has proved to be challenging, as both *Giardia* and *Cryptosporidium* form dormant and chemically resistant structures called cysts during their life cycles. The cyst forms are resistant to the killing action of chlorine and can pass through the filters typically used in water treatment plants. Contamination of the water supply of Milwaukee, Wisconsin with *Cryptosporidium* in 1993 sickened over 400,000 people and the deaths of at least 47 people were subsequently attributed to the contamination.

Water quality testing often involves the use of a test that measures the turbidity of the water. Turbidity gives an indication of the amount of particulate material in the water. If the water is contaminated with particles as small as bacteria and viruses, the turbidity of the water will increase. Thus, the turbidity test can be a quick means of assessing if water quality is deteriorating and whether further action should be taken to enhance the quality of the water supply.

Water quality is also addressed in many countries by regulations that require the sampling and testing of drinking water for microorganisms. Testing is typically for an "indicator" of fecal pollution of the water. *Escherichia coli* is often the most suitable indicator organism. The bacterium is present in the intestinal tract in greater numbers than the disease-causing bacteria and viruses. Thus, the chances of detecting the indicator organism is better than detecting the actual pathogen. Additionally, the indicator does not usually multiply in the water (except in tropical countries), so its presence is indicative of recent fecal pollution. Finally, *Escherichia coli* can be detected using tests that are inexpensive and easy to perform.

Because the prevention of water borne disease rests on the adequate treatment of the water, underdeveloped regions of the world continue to experience the majority of water borne diseases. For example, in India the prevalence of cholera is so great that the disease is considered to be epidemic. But, as exemplified by communities like Walkerton and Milwaukee, even developed countries having an extensive water treatment infrastructure can experience problems if the treatment barriers are breached by the microorganisms.

See also Bacteria and bacterial infection; Bioremediation; Epidemics and pandemics; Water purification

WATSON, JAMES D. (1928-)

American molecular biologist

James D. Watson won the 1962 Nobel Prize in physiology and medicine along with **Francis Crick** and Maurice Wilkins for discovering the structure of **DNA**, or **deoxyribonucleic acid**, the molecular carrier of genetic information. Watson and Crick had worked as a team since meeting in the early 1950s, and their research ranks as a fundamental advance in **molecular biology**.

James Dewey Watson was born in Chicago, Illinois, on April 6, 1928, to James Dewey and Jean (Mitchell) Watson. He was educated in the Chicago public schools, and during his adolescence became one of the original Quiz Kids on the radio show of the same name. Shortly after this experience in 1943, Watson entered the University of Chicago at the age of 15.

Watson graduated in 1946, but stayed on at Chicago for a bachelor's degree in zoology, which he attained in 1947. During his undergraduate years Watson studied neither genetics nor biochemistry—his primary interest was in the field of ornithology. In 1946, Watson spent a summer working on advanced ornithology at the University of Michigan's summer research station at Douglas Lake. During his undergraduate career at Chicago, Watson had been instructed by the well-known population geneticist Sewall Wright, but he did not become interested in the field of genetics until he read Erwin Schrödinger's influential book *What Is Life?* It was then, Horace Judson reports in *The Eighth Day of Creation: Makers of the Revolution in Biology,* that Watson became interested in finding out the secret of the **gene**.

Watson enrolled at Indiana University to perform graduate work in 1947. Indiana had several remarkable geneticists who could have been important to Watson's intellectual development, but he was drawn to the university by the presence of the Nobel laureate Hermann Joseph Muller, who had demonstrated 20 years earlier that x rays cause mutation. Nonetheless, Watson chose to work under the direction of the Italian biologist Salvador Edward Luria, and it was under Luria that he began his doctoral research in 1948.

Watson's thesis was on the effect of x rays on the rate of phage lysis (a phage, or **bacteriophage**, is a bacterial virus). The biologist Max Delbrück and Luria—as well as a number of others who formed what was to be known as "the phage group"—demonstrated that phages could exist in a number of mutant forms. A year earlier Luria and Delbrück had published one of the landmark papers in **phage genetics**, in which they established that one of the characteristics of phages is that they can exist in different genetic states so that the lysis (or bursting) of bacterial host cells can take place at different rates. Watson's Ph.D. degree was received in 1950, shortly after his twenty-second birthday.

Watson was next awarded a National Research Council fellowship grant to investigate the molecular structure of proteins in Copenhagen, Denmark. While Watson was studying enzyme structure in Europe, where techniques crucial to the study of macromolecules were being developed, he was also attending conferences and meeting colleagues.

From 1951 to 1953, Watson held a research fellowship under the support of the National Foundation for Infantile Paralysis at the Cavendish Laboratory in Cambridge, England. Those two years are described in detail in Watson's 1965 book, *The Double Helix: A Personal Account of the Discovery of the Structure of DNA.* An autobiographical work, *The Double Helix* describes the events—both personal and professional—that led to the discovery of DNA. Watson was to work at the Cavendish under the direction of Max Perutz, who was engaged in the x-ray crystallography of proteins. However, he soon found himself engaged in discussions with Crick on the structure of DNA. Crick was 12 years older than Watson and, at the time, a graduate student studying protein structure.

Intermittently over the next two years, Watson and Crick theorized about DNA and worked on their model of DNA structure, eventually arriving at the correct structure by recognizing the importance of x-ray diffraction photographs pro-

duced by Rosalind Franklin at King's College, London. Both were certain that the answer lay in model-building, and Watson was particularly impressed by Nobel laureate Linus Pauling's use of model-building in determining the alpha-helix structure of protein. Using data published by Austrian-born American biochemist Erwin Chargaff on the symmetry between the four constituent nucleotides (or bases) of DNA molecules, they concluded that the building blocks had to be arranged in pairs. After a great deal of experimentation with their models, they found that the double helix structure corresponded to the empirical data produced by Wilkins, Franklin, and their colleagues. Watson and Crick published their theoretical paper in the journal *Nature* in 1953 (with Watson's name appearing first due to a coin toss), and their conclusions were supported by the experimental evidence simultaneously published by Wilkins, Franklin, and Raymond Goss. Franklin died in 1958. Wilkins shared the Nobel Prize with Watson and Crick in 1962.

After the completion of his research fellowship at Cambridge, Watson spent the summer of 1953 at Cold Spring Harbor, New York, where Delbrück had gathered an active group of investigators working in the new area of molecular biology. Watson then became a research fellow in biology at the California Institute of Technology, working with Delbrück and his colleagues on problems in phage genetics. In 1955, he joined the biology department at Harvard and remained on the faculty until 1976. While at Harvard, Watson wrote *The Molecular Biology of the Gene* (1965), the first widely used university textbook on molecular biology. This text has gone through seven editions, and now exists in two large volumes as a comprehensive treatise of the field. In 1968, Watson became director of Cold Spring Harbor, carrying out his duties there while maintaining his position at Harvard. He gave up his faculty appointment at the university in 1976, however, and assumed full-time leadership of Cold Spring Harbor. With John Tooze and David Kurtz, Watson wrote *The Molecular Biology of the Cell,* originally published in 1983.

In 1989, Watson was appointed the director of the Human Genome Project of the National Institutes of Health, but after less than two years he resigned in protest over policy differences in the operation of this massive project. He continues to speak out on various issues concerning scientific research and is a strong presence concerning federal policies in supporting research. In addition to sharing the Nobel Prize, Watson has received numerous honorary degrees from institutions and was awarded the Presidential Medal of Freedom in 1977 by President Jimmy Carter. In 1968, Watson married Elizabeth Lewis. They have two children.

In his book, *The Double Helix*, Watson confirms that never avoided controversy. His candor about his colleagues and his combativeness in public forums have been noted by critics. On the other hand, his scientific brilliance is attested to by Crick, Delbrück, Luria, and others. The importance of his role in the DNA discovery has been well supported by Gunther Stent—a member of the Delbrück phage group—in an essay that discounts many of Watson's critics through well-reasoned arguments.

Most of Watson's professional life has been spent as a professor, research administrator, and public policy spokesman for research. More than any other location in Watson's professional life, Cold Spring Harbor (where he is still director) has been the most congenial in developing his abilities as a scientific catalyst for others. Watson's work there has primarily been to facilitate and encourage the research of other scientists.

James Watson, co-discoverer of the structure of the DNA double helix.

See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; DNA (Deoxyribonucleic acid); DNA chips and micro arrays; DNA hybridization; Genetic code; Genetic identification of microorganisms; Genetic mapping; Genetic regulation of eukaryotic cells; Genetic regulation of prokaryotic cells; Genotype and phenotype; Molecular biology and molecular genetics

WELCH, WILLIAM HENRY (1850-1934)

American pathologist

William Henry Welch was a senior pathologist at Johns Hopkins University and its hospital. He researched numerous diseases, including **pneumonia** and **diphtheria**, but is most renowned for his discovery of the *Bacillus welchii,* a bacterium that causes gangrene. Throughout his career, Welch

advocated asepsis and other general reforms in American hospitals to control disease and advance medical care.

Welch was born in Norfolk, Connecticut in 1850. He attended Yale and graduated in 1870. He then studied to be a surgeon at Columbia University, earning his M.D. in 1875. Welch then pursued advanced studies in Europe. He studied at several universities, but was perhaps most influenced by his time in Berlin. He returned to the United States in 1878 and was a professor and physician at Bellevue Hospital and Medical College in New York.

Welch conducted most of his career research as a professor and pathologist-in-chief at Johns Hopkins University and hospital. He accepted a position at the emerging hospital and medical school in 1884. His commitment to hospital reform and **public health** led to his discovery of the cause of gas gangrene. Later, Welch was named the director of the School of **Hygiene** and Public Health.

Welch's commitment to public health, as well as clinical medicine, garnered several awards, including the U.S. Army Distinguished Service Medal and Citation. Because gangrene was not only a serious surgical risk, but also an endemic problem with battle wounds, Welch's identification of *Bacillus welchii* was of military and medical interest.

In addition to his academic appointments, Welch held several offices in professional organizations. He founded the *Journal of Experimental Medicine* in 1896. Welch served on the Maryland State Board of Health for 31 years. He was president of the American Medical Association in 1910.

Welch died in 1934, while still serving on several medical boards.

See also Bacteria and bacterial infection; History of microbiology

WELLER, THOMAS (1915-)

American physician

Thomas Weller was corecipient, with **John F. Enders** and Frederick Robbins, of the Nobel Prize in physiology or medicine in 1954. This award was given for the trio's successful growth of the **poliomyelitis** (polio) virus in a non-neural tissue **culture**. This development was significant in the fight against the crippling disease polio, and eventually led to the development, by **Jonas Salk** in 1953, of a successful **vaccination** against the virus. It also revolutionized viral work in the laboratory and aided the recognition of many new **types of viruses**. Weller also distinguished himself with his studies of human **parasites** and the **viruses** that cause rubella and chickenpox.

Thomas Huckle Weller was born June 15, 1915, in Ann Arbor, Michigan. His parents were Elsie A. (Huckle) and Dr. Carl V. Weller. He received his B.S. in 1936 and M.S. in 1937, both from the University of Michigan, where his father was chair of the pathology department. He continued his studies at Harvard Medical School, where he met and roomed with his future Nobel corecipient Robbins. In 1938, Weller received a fellowship from the international health division of the Rockefeller Foundation, which allowed him to study **public health** in Tennessee and **malaria** in Florida, topics which first interested him during his undergraduate years.

Weller graduated from Harvard with magna cum laude honors in parasitology, receiving his M.D. in 1940. He also received a fellowship in tropical medicine and a teaching fellowship in bacteriology. He completed an internship in pathology and bacteriology (1941) at Children's Hospital in Boston. He then began a residency at Children's, with the intention of specializing in pediatrics, before enlisting in the U.S. Army during World War II.

Weller served in the Army Medical Corps from 1942 to 1945. He was initially given teaching assignments in tropical medicine, but he was soon made officer in charge of bacteriology and **virology** work in San Juan, Puerto Rico. His major research there related to **pneumonia** and the parasitic disease schistosomiasis, an infection that is centered in the intestine and damages tissue and the circulatory system. Before his military service ended, he moved to the Army Medical School in Washington D.C. Upon his discharge in 1945, Weller was married to Kathleen Fahey, with whom he had two sons and two daughters. Returning to Boston's Children's Hospital, he finished his residency and began a post-doctoral year working with Enders.

During 1948, Weller was working with the **mumps** virus, which Enders had been researching since the war. After one experiment, Weller had a few tubes of human embryonic tissue left over, so he and Enders decided to see what the virus poliomyelitis might do in them. A small amount of success prompted the duo, who had been joined in their research by Robbins, to try growing the virus in other biological mediums, including human foreskin and the intestinal cells of a mouse. The mouse intestine did not produce anything, but the trio finally had significant viral growth with human intestinal cells. This was the first time poliomyelitis had been grown in human or simian tissue other than nerve or brain. Using **antibiotics** to ward off unwanted bacterial invasion, the scientists were able to isolate the virus for study.

Once poliomyelitis was grown and isolated in tissue cultures it was possible to closely study the nature of the virus, which in turn made it possible for Salk to create a **vaccine** in 1953. Besides leading to an inhibitor against a debilitating disease, a major result of the trio's development was a decrease in the need for laboratory animals. As Weller was quoted saying in the *Journal of Infectious Diseases,* "In the instance of poliomyelitis, one culture tube of human or monkey cells became the equivalent of one monkey." In times prior, viruses had to be injected into living animals to monitor their potency. Now, with tissue culture growth, cell changes were apparent under the **microscope**, showing the action of the virus and eliminating the need for the animals. The techniques for growing cells in tissue cultures developed by Weller and his associates were not only applicable to the poliomyelitis virus, however. They were soon copied by many other labs and scientists and quickly led to the identification, control, and study of several previously unrecognized virus types. For their work, and the improvements in scientific research it made possible, Weller, Enders, and Robbins shared the 1954 Nobel Prize in physiology or medicine.

Concurrent with his work with Enders and Robbins, Weller was named assistant director of the research division of infectious diseases at Children's Hospital in 1949. He held this position until 1954. At the same time, he began teaching at Harvard in tropical medicine and tropical public health, moving from instructor to associate professor. In 1953, Weller and Robbins shared the Mead Johnson Prize for their contributions to pediatric research. Then, in 1954, Weller was named Richard Pearson Strong Professor of Tropical Public Health and chair of the public health department at Harvard. As a consequence, he moved his research facilities to the Harvard Medical School. Later, he was appointed director of the Center for Prevention of Infectious Diseases at the Harvard School of Public Health.

From the end of World War II until 1982, Weller also continued his research on two types of helminths, *trichinella spiralis* and *schistosoma mansoni*. Helminths are intestinal parasites, and these two cause, respectively, trichinosis, which can also severely affect the human musculature, and schistosomiasis. Weller was concerned with the parasites' basic biology and performed various diagnostic studies on them. His contributions to current understanding of these parasites are significant, advancing an understanding of the ailments they cause.

Weller spent a portion of the same period (1957 to 1973) establishing the basic available knowledge concerning cytomegalovirus (commonly known as CMV), which causes cell enlargement in various organs. Weller's most important finding in this area regarded congenital transmission of both CMV and rubella, a virus also known as German **measles**. A pregnant woman infected with either of these viruses may pass the infection on to her fetus. Weller showed that infected newborns excreted viral strains in their feces, providing another source for the spread of the diseases. His findings became significant when it was also learned that children born to infected mothers often risked birth defects.

In 1962, Weller, along with Franklin Neva, was able to grow and study German Measles in tissue cultures. These two also went on to grow and isolate the chickenpox virus. Subsequently, Weller was the first to show the common origin of the **varicella** virus, which causes chicken pox, and the **herpes** zoster virus, which causes shingles. In 1971, Weller was the first to prove the airborne transmission of *pneumocystis carinii*, a form of pneumonia that later appeared as a frequent side effect of the **human immunodeficiency virus** commonly known as **HIV**.

Weller was elected to the National Academy of Sciences in 1964. In addition, he served on advisory committees of the **World Health Organization**, the Pan American Health Organization, the Agency for International Development, and the National Institute of Allergy and Infectious Disease. He continued his position at Harvard until 1985, when he became professor emeritus. While at Harvard, he helped establish the Public Health Department's international reputation. In 1988, Weller gave the first John F. Enders Memorial Lecture to the Infectious Disease Society of America. In addition to his Nobel Prize, Weller was the recipient of many awards and honorary degrees during his career.

See also Laboratory techniques in immunology; Virology; Virus replication; Viruses and responses to viral infection

West Nile virus

The West Nile virus is a member of the family Flaviviridae, a virus that has become more prominent in Europe and North America in the past decade. The virus, which is closely related to the St. Louis encephalitis virus found in the United States, causes an encephalitis (swelling of the brain) in domestic animals (such as horses, dogs, cats), wild animals, and wild birds. When transferred from an infected animal to a human, the viral infection can produce encephalitis as well as **inflammation** of nerve cells of the spinal cord (**meningitis**).

In 1937, the virus was isolated from a woman in the West Nile District of Uganda. This locale was the basis for the designation of the virus as the West Nile virus. During the 1950s, the ability of the virus to cause the serious and life-threatening human disease was recognized. In the 1960s, the virus was established as a cause of equine encephalitis.

Whether the virus has spread geographically from Uganda, or whether increased surveillance has detected the virus in hitherto unsuspected regions is not clear. However, the pattern of detection has been that of a global dissemination. Long found in humans, animals, and birds in Africa, Eastern Europe, West Asia, and the Middle East, the virus was first detected in North America in 1999.

The virus has come to prominent attention in North America following its 1999 appearance on the continent. That year, 62 cases of the disease were reported in New York City. Seven people died. The following year 21 more cases occurred, and two of the people died. In 1999 and 2000, the West Nile virus was confined to the northeastern coastal states of the United States. However, an inexorable spread to other regions of the country and the continent has begun. In the summer of 2001, dead birds that tested positive for the virus were found as far north as Toronto, Canada, as far south as the northern portion of Florida, and as far west as Milwaukee, Wisconsin. Scientists anticipate that the virus will continue to disseminate. During the summer of 2002, more than 300 cases and at least 14 deaths were reported—with a continued spread of the virus into the western United States. By August 2002, West Nile virus was reported in 41 states.

The mosquitoes are the prime vector of the West Nile virus. When mosquitoes obtain a blood meal from an infected animal or a bird, they acquire the virus. The virus resides in the salivary glands of the mosquito, to be passed on to a human when the mosquito seeks another blood meal. The cases in New York City, especially those in 2000, are thought to have been caused by the bite of virus-infected mosquitoes that survived the cold winter months. The emergence of the mosquito in the spring can facilitate the re-emergence of the virus. For example in North America, there were large die-offs of crow populations due to West Nile virus in the Spring of 2000 and then again in the Spring of 2001.

Upon entry to a host's bloodstream, multiplication of the virus in the blood occurs. Then, by a mechanism that is not yet deciphered, the virus crosses the barrier between the blood and the brain. Subsequent multiplication of the virus in brain tissue causes nervous system malfunction and inflammation of the infected brain tissue.

Although a large population of mosquitoes may be present, the chances of acquiring West Nile virus via a mosquito bite is small. Data from the examination of mosquito populations indicates that less than one percent of mosquitoes carry the virus, even in areas where the virus is known to be present.

The mosquito to human route of infection is the only route known thus far. The virus is known to infect certain species of ticks. However, as of early 2002, tick-borne outbreak of the disease has not been documented in humans. Person to person contact cannot occur. Even exchange of body fluids between an infected human and an uninfected person will not transmit the virus.

Currently no human **vaccine** to the West Nile virus exists. Prevention of infection consists of repelling mosquitoes by conventional means, such as the use of repellent sprays or creams, protective clothing, and avoiding locations or times of the day or season when mosquitoes might typically be encountered.

See also Viruses and responses to viral infection; Zoonoses

WET MOUNT • *see* MICROSCOPE AND MICROSCOPY

WHOOPING COUGH • *see* PERTUSSIS

WILKINS, MAURICE HUGH FREDERICK (1916-)

New Zealand English biophysicist

Maurice Hugh Frederick Wilkins is best known for his work regarding the discovery of the structure of **deoxyribonucleic acid** (**DNA**). Along with American molecular biologist **James D. Watson** (1924–) and English molecular biologist **Francis Crick** (1916–), Wilkins received the 1962 Nobel Prize in physiology or medicine for his contributions to the discovery of the molecular mechanisms underlying the transmission of genetic information. Specifically, Wilkins' contribution involved discerning the structure of DNA through the use of x–ray diffraction techniques.

Wilkins was born in Pongaroa, New Zealand to Irish immigrants Edgar Henry, a physician, and Eveline Constance Jane (Whittaker) Wilkins. Euperior education began at an early age for Wilkins, who began attending King Edward's School in Birmingham, England, at age six. He later received his B.A. in physics from Cambridge University in 1938. After graduation, he joined the Ministry of Home Security and Aircraft Production and was assigned to conduct graduate research on radar at the University of Birmingham. Wilkins' research centered on improving the accuracy of radar screens.

Soon after earning his Ph.D. in 1940, Wilkins, still with the Ministry of Home Security, was relocated to a new team of British scientists researching the application of uranium isotopes to atomic bombs. A short time later Wilkins became part of another team sent to the United States to work on the Manhattan Project—the military effort to develop the atomic bomb—with other scientists at the University of California at Berkeley. He spent two years there researching the separation of uranium isotopes.

Wilkins' interest in the intersection of physics and biology emerged soon after his arrival to the United States. He was significantly influenced by a book by Erwin Schrödinger, a fellow physicist, entitled *What is Life? The Physical Aspects of the Living Cell*. The book centers on the possibility that the science of quantum physics could lead to the understanding of the essence of life itself, including the process of biological growth. In addition to Schrödinger's book, the undeniable and undesirable ramifications of his work on the atomic bomb also played a role in Wilkins' declining interest in the field of nuclear physics and emerging interest in biology.

After the war, the opportunity arose for Wilkins to begin a career in biophysics. In 1945, Wilkins' former graduate school professor, Scottish physicist John T. Randall, invited him to become a physics lecturer at St. Andrews University, Scotland, in that school's new biophysics research unit. Later, in 1946, Wilkins and Randall moved on to a new research pursuit combining the sciences of physics, chemistry, and biology to the study of living cells. Together they established the Medical Research Council Biophysics Unit at King's College in London. Wilkins was, for a time, informally the second in command. He officially became deputy director of the unit in 1955 and was promoted to director in 1970, a position he held until 1972.

It was at this biophysics unit, in 1946, that Wilkins soon concentrated his research on DNA, shortly after scientists at the Rockefeller Institute (now Rockefeller University) in New York announced that DNA is the constituent of genes. Realizing the enormous importance of the DNA molecule, Wilkins became excited about uncovering its precise structure. He was prepared to attack this project by a number of different methods. However, he fortuitously discovered that the particular makeup of DNA, specifically the uniformity of its fibers, made it an excellent specimen for x–ray diffraction studies. x–ray diffraction is an extremely useful method for photographing atom arrangements in molecules. The regularly–spaced atoms of the molecule actually diffract the x rays, creating a picture from which the sizing and spacing of the atoms within the molecule can be deduced. This was the tool used by Wilkins to help unravel the structure of DNA.

Physical chemist Rosalind Franklin joined Wilkins in 1951. Franklin, who had been conducting research in Paris, was adept in x–ray diffraction. Together they were able to retrieve some very high quality DNA patterns. One initial and important outcome of their research was that phosphate groups were located outside of the structure, which overturned Linus Pauling's theory that they were on the inside. In another important finding, Wilkins thought the photographs suggested a helical structure, although Franklin hesitated to draw that conclusion. Subsequently, Wilkins passed on to Watson one of the best x–ray pictures Franklin had taken of DNA. These DNA images provided clues to Watson and Crick, who used the pictures to solve the last piece of the DNA structure puzzle.

Consequently, in 1953, Watson and Crick were able to reconstruct the famous double–helix structure of DNA. Their model shows that DNA is composed of two strands of alternating units of sugar and phosphate on the outside, with pairs

of bases—including the molecular compounds adenine, thymine, guanine, and cytosine—inside, bonded by hydrogen. It is important to note that while Wilkins' contribution to the discernment DNA's structure is undeniable, controversy surrounds how Watson and Crick obtained Franklin's photographs and the fact that Franklin was not recognized for this scientific breakthrough, particularly in terms of the Nobel Prize. Because the Nobel Prize is not awarded posthumously, Franklin, who died of cancer in 1958, did not receive the same recognition as did Watson, Crick, and Wilkins.

The knowledge of the DNA structure, which has been described as resembling a spiral staircase, has provided the impetus for advanced research in the field of genetics. For example, scientists can now determine predispositions for certain diseases based on the presence of certain genes. Also, the exciting but sometimes controversial area of genetic engineering has developed.

Wilkins, Watson, and Crick were awarded the 1962 Nobel Prize for physiology or medicine for their work which uncovered the structure of hereditary material DNA. After winning the Nobel Prize, Wilkins focused next on elucidating the structure of ribonucleic acids (RNA)—a compound like DNA associated with the control of cellular chemical activities—and, later, nerve cell membranes. In 1962, Wilkins was able to show that **RNA** also had a helical structure somewhat similar to that of DNA. Besides his directorship appointments at the Medical Research Council's Biophysics Unit, Wilkins was also appointed director of the Council's Neurobiology Unit, a post he held from 1974 to 1980. Additionally, he was a professor at King's College, teaching **molecular biology** from 1963 to 1970, and then biophysics as the department head from 1970 to 1982. In 1981, he was named professor emeritus at King's College. Utilizing some of his professional expertise for social causes, Wilkins has maintained membership in the British Society for Social Responsibility in Science (of which he is president), the Russell Committee against Chemical Weapons, and Food and Disarmament International.

Wilkins is an honorary member of the American Society of Biological Chemists and the American Academy of Arts and Sciences. He was also honored with the 1960 Albert Lasker Award of the American Public Health Association (given jointly to Wilkins, Watson, and Crick), and was named Fellow of the Royal Society of King's College in 1959.

See also DNA (Deoxyribonucleic acid); DNA chips and micro arrays; Gene; Genetic mapping; Molecular biology and molecular genetics

WINE MAKING

Along with bread making, the use of the **microorganisms** called yeasts to produce wine from grapes is one of the oldest uses of microorganisms by man. The origins of wine making date from antiquity. Before 2000 B.C. the Egyptians would store crushed fruit in a warm place in order to produce a liquid whose consumption produced feelings of euphoria. The manufacture and consumption of wine rapidly became a part of daily life in many areas of the Ancient world and eventually became a well-established part of Classical civilization. For centuries, wine making has been an important economic activity. In certain areas of the world, such as France, Italy, and Northern California, wine making on a commercial scale is a vital part of the local economy.

The agent of the formation of wine is **yeast**. Yeasts are small, single-celled **fungi** that belong to the genus Ascomycota. Hallmarks of yeast are their ability to reproduce by the methods of fission or budding, and their ability to utilize compounds called carbohydrates (specifically the sugar glucose) with the subsequent production of alcohol and the gas carbon dioxide. This chemical process is called **fermentation**.

Yeast cells are able to carry out fermentation because of **enzymes** they possess. The conversion of sugar to alcohol ultimately proves lethal to the yeast cells, which cannot tolerate the increasing alcohol levels. Depending on the type of yeast used, the alcohol content of the finished product can vary from around 5% to over 20%, by volume.

The scientific roots of fermentation experimentation date back to the seventeenth century. In 1680 **Anton van Leeuwenhoek** used his hand-built light microscopes to detect yeast. Almost one hundred years later the French chemist Antoine Laurent Lavoisier proposed that yeast was the agent of the fermentation of sugar. This was confirmed in 1935 by the examination of yeast vats with the greatly improved microscopes of that day.

In the nineteenth century the role of yeasts as a catalyst (that is, as an agent that accelerates a chemical process without itself being changed in the process) was recognized by the Swedish chemist Jons Berzelius. In the 1860s the renowned microbiologist **Louis Pasteur** discovered that yeast fermentation could proceed in the absence of oxygen. In 1878 Wilhelm Kuhne recognized that the yeast catalyst was contained inside the cell. He coined the term "enzyme" for the catalyst.

In fact more than two dozen yeast enzymes participate in the degradation of glucose. The degradation is a pathway, with one reaction being dependent on the occurrence of a prior reaction, and itself being required for a subsequent reaction. In total some 30 chemical reactions are involved. These reactions require the function of the various enzymes. The yeast cell is the biological machine that creates the enzymes. Once the enzymes are present, alcoholic fermentation can proceed in the absence of living yeast. Enzymes, however, have only a finite period of activity before they themselves degrade. Hence a continual supply of fresh enzymes requires living yeast.

Many types of yeast exist. The stable types suitable for making wine (and bread and beer) are the seven species of yeast belonging to the genus Saccharomyces. The name comes from the Greek words for sugar (sacchar) and fungus (Mykes). The predominant species in wine making is *Saccharomyces cerevisiae*. There are multiple strains of this species that produce wine. The **selection** of yeast type is part of the art of wine making; the yeast is matched to the grape and the fermentation conditions to produce—the wine maker hopes—a finished product of exceptional quality.

The natural source of yeast for wine making is often the population that becomes dominant in the vineyard. Less

Barrels used to age wine in the wine making process.

mature local vineyards, especially those established in North America, rely on yeast strains that are injected into the crushed grape suspension. The growth of the yeast will then occur in the nutrient-rich mixture of the suspension.

The fermentation process begins when the yeast is added to the juice that is obtained following the crushing of the grapes. This process can be stunted or halted by the poor growth of the yeast. This can occur if conditions such as temperature and light are not favorable. Also, contaminating microorganisms can outgrow the yeast and out compete the yeast cells for the nutrients. Selective growth of *Sacchromyces cerevisiae* can be encouraged by maintaining a temperature of between 158 and 167°F (70 and 75°C). The **bacteria** that are prone to develop in the fermenting suspension do not tolerate such an elevated temperature. Yeast other than *Sacchromyces cerevisiae* are not as tolerant of the presence of sulfur dioxide. Thus the addition of compounds containing sulfur dioxide to fermenting wine is a common practice.

The explosion in popularity of home-based wine making has streamlined the production process. Home vintners can purchase so-called starter yeast, which is essentially a powder consisting of a form of the yeast that is dormant. Upon the addition of the yeast powder to a solution of grape essence and sugar, resuscitation of the yeast occurs, growth resumes, and fermentation starts. In another modification to this process, the yeast starter can be added to a liquid growth source for a few days. Then this new **culture** of yeast can be used to inoculate the grape essence and sugar solution. The advantage of the second approach is that the amount of yeast, which is added, can be better controlled, and the addition of liquid culture encourages a more efficient dispersion of the yeast cells throughout the grape solution.

The many varieties of wine, including champagne, are the results of centuries of trial and error involving the myriad varieties of grape and yeast.

See also Economic uses and benefits of microorganisms; Fermentation

WINOGRADSKY COLUMN

In a Winogradsky column the conditions change from oxygen-rich (aerobic) at the top of the column to oxygen-deficient (anaerobic) at the bottom. Different **microorganisms** develop in the various environmental niches throughout the column. The products of one microbe's metabolic activities support the growth of another microbe. The result is that the column becomes a self-supporting ecosystem, which is driven only by the energy received from the incoming sunlight. Winogradsky columns are easily constructed, and are often used in classroom experiments and demonstrations.

The Winogradsky column is named after Sergius Winogradsky, a Russian microbiologist who was one of the pioneers of the study of the diversity of the metabolic activities of microorganisms.

To set up a Winogradsky column, a glass or clear plastic tube is filled one-third full with a mixture of mud obtained from a river bottom, cellulose, sodium sulphate, and calcium carbonate. The remaining two-thirds of the tube is filled with lake or river water. The capped tube is placed near a sunlit window.

Over a period of two to three months, the length of the tube becomes occupied by a series of microbial communities. Initially, the cellulose provides nutrition for a rapid increase in bacterial numbers. The growth uses up the available oxygen in the sealed tube. Only the top water layer continues to contain oxygen. The sediment at the bottom of the tube, which has become completely oxygen-free, supports the growth only of those **bacteria** that can grow in the absence of oxygen. Desulfovibrio and Clostridium will predominate in the sediment.

Diffusion of hydrogen sulfide produced by the anaerobic bacteria, from the sediment into the water column above supports the growth of anaerobic photosynthetic bacteria such as green sulfur bacteria and purple sulfur bacteria. These bacteria are able to utilize sunlight to generate energy and can use carbon dioxide in a oxygen-free reaction to produce compounds needed for growth.

The diminished hydrogen sulfide conditions a bit further up the tube then support the development of purple sulfur bacteria such as Rhodopseudomonas, Rhodospirillum, and Rhodomicrobium.

Towards the top of the tube, oxygen is still present in the water. Photosynthetic cyanobacteria will grow in this region, with the surface of the water presenting an atmosphere conducive to the growth of **sheathed bacteria**.

The Winogradsky column has proved to be an excellent learning tool for generations of microbiology students, and a classic demonstration of how carbon and energy specifics result in various niches for different microbes, and of the recycling of sulfur, nitrogen, and carbon.

Flossie Wong-Staal, a pioneer in AIDS research.

See also Chemoautotrophic and chemolithotrophic bacteria; Methane oxidizing and producing bacteria

WONG-STAAL, FLOSSIE (1947-)

Chinese American virologist

Although Flossie Wong-Staal is considered one of the world's top experts in **viruses** and a codiscoverer of the **human immunodeficiency virus** (**HIV**) that causes **AIDS**, her interest in science did not come naturally.

Born as Yee Ching Wong in communist mainland China, she fled with her family in 1952 to Hong Kong, where she entered an all-girls Catholic school. When students there achieved high grades, they were steered into scientific studies. The young Wong had excellent marks, but initially had no plans of becoming a scientist. Against her expectations, she gradually became enamored with science. Another significant result of attending the private school was the changing of her name. The school encouraged Wong to adopt an English name. Her father, who did not speak English, chose the name Flossie from newspaper accounts of Typhoon Flossie, which had struck Hong Kong the previous week.

Even though none of Wong's female relatives had ever gone to college or university, her family enthusiastically supported her education and in 1965, she went to the United States to study at the University of California at Los Angeles. In 1968, Wong graduated magna cum laude with a B.S. in bacteriology, also obtaining a doctorate in **molecular biology** in 1972.

During postgraduate work at the university's San Diego campus in 1971–72, Wong married and added Staal to her name. The marriage eventually ended in divorce. In 1973,

Wong-Staal moved to Bethesda, Maryland, where she worked at the National Cancer Institute (NCI) with AIDS pioneer Robert Gallo, studying **retroviruses**, the mysterious family of viruses to which HIV belongs. Searching for a cause for the newly discovered AIDS epidemic, Gallo, Wong-Staal, and other NCI colleagues identified HIV in 1983, simultaneously with a French researcher. In 1985, Wong-Stall was responsible for the first **cloning** of HIV. Her efforts also led to the first **genetic mapping** of the virus, allowing eventual development of tests that screen patients and donated blood for HIV.

In 1990, the Institute for Scientific Information declared Wong-Staal as the top woman scientist of the previous decade. That same year, Wong-Staal returned to the University of California at San Diego to continue her AIDS research. Four years later, the university created a new Center for AIDS Research; Wong-Staal became its chairman. There, she works to find both vaccines against HIV and a cure for AIDS, using the new technology of **gene** therapy.

See also AIDS, recent advances in research and treatment

WOODWARD, ROBERT B. (1917-1979)

American biochemist

Robert B. Woodward was arguably the greatest organic synthesis chemist of the twentieth century. He accomplished the total synthesis of several important natural products and pharmaceuticals. Total synthesis means that the molecule of interest—no matter how complex—is built directly from the smallest, most common compounds and is not just a derivation of a related larger molecule. In order to accomplish his work, Woodward combined physical chemistry principles, including quantum mechanics, with traditional reaction methods to design elaborate synthetic schemes. With Nobel Laureate Roald Hoffmann, he designed a set of rules for predicting reaction outcomes based on stereochemistry, the study of the spatial arrangements of molecules. Woodward won the Nobel Prize in chemistry in 1965.

Robert Burns Woodward was born in Boston on April 10, 1917, to Arthur and Margaret (Burns) Woodward. His father died when he was very young. Woodward obtained his first chemistry set while still a child and taught himself most of the basic principles of the science by doing experiments at home. By the time he graduated at the age of 16 from Quincy High School in Quincy, Massachusetts, in 1933, his knowledge of chemistry exceeded that of many of his instructors. He entered the Massachusetts Institute of Technology (MIT) the same year but nearly failed a few months later, apparently impatient with the rules and required courses.

The MIT chemistry faculty, however, recognized Woodward's unusual talent and rescued him. They obtained funding and a laboratory for his work and allowed him complete freedom to design his own curriculum, which he made far more rigorous than the required one. Woodward obtained his doctorate degree from MIT only four years later, at the age of 20, and then joined the faculty of Harvard University after a year of postdoctoral work there.

Woodward spent virtually all of his career at Harvard but also did a significant amount of consulting work with various corporations and institutes around the world. As is true in most modern scientific endeavors, Woodward's working style was characterized by collaboration with many other researchers. He also insisted on utilizing the most up-to-date instrumentation, theories.

The design of a synthesis, the crux of Woodward's work, involves much more than a simple list of chemicals or procedures. Biochemical molecules exhibit not only a particular bonding pattern of atoms, but also a certain arrangement of those atoms in space. The study of the spatial arrangements of molecules is called stereochemistry, and the individual configurations of a molecule are called its stereoisomers. Sometimes the same molecule may have many different stereoisomers; only one of those, however, will be biologically relevant. Consequently, a synthesis scheme must consider the basic reaction conditions that will bond two atoms together as well as determine how to ensure that the reaction orients the atoms properly to obtain the correct stereoisomer.

Physical chemists postulate that certain areas around an atom or molecule are more likely to contain electrons than other areas. These areas of probability, called orbitals, are described mathematically but are usually visualized as having specific shapes and orientations relative to the rest of the atom or molecule. Chemists visualize bonding as an overlap of two partially full orbitals to make one completely full molecular orbital with two electrons. Woodward and Roald Hoffmann of Cornell University established the Woodward-Hoffmann rules based on quantum mechanics, which explain whether a particular overlap is likely or even possible for the orbitals of two reacting species. By carefully choosing the shape of the reactant species and reaction conditions, the chemist can make certain that the atoms are oriented to obtain exactly the correct stereochemical configuration. In 1970, Woodward and Hoffmann published their classic work on the subject, *The Conservation of Orbital Symmetry;* Woodward by that time had demonstrated repeatedly by his own startling successes at synthesis that the rules worked.

Woodward and his colleagues synthesized a lengthy list of difficult molecules over the years. In 1944 their research, motivated by wartime shortages of the material and funded by the Polaroid Corporation, prompted Woodward—only 27 years old at the time—and William E. Doering to announce the first total synthesis of quinine, important in the treatment of **malaria**. Chemists had been trying unsuccessfully to synthesize quinine for more than a century.

In 1947, Woodward and C. H. Schramm, another organic chemist, reported that they had created an artificial protein by bonding amino acids into a long chain molecule, knowledge that proved useful to both researchers and workers in the plastics industry. In 1951, Woodward and his colleagues (funded partly by Merck and the Monsanto Corporation) announced the first total synthesis of cholesterol and cortisone, both biochemical steroids. Cortisone had only recently been identified as an effective drug in the treatment of rheumatoid arthritis, so its synthesis was of great importance.

Woodward's other accomplishments in synthesis include strychnine (1954), a poison isolated from *Strychnos*

species and often used to kill rats; colchicine (1963), a toxic natural product found in autumn crocus; and lysergic acid (1954) and reserpine (1956), both psychoactive substances. Reserpine, a tranquilizer found naturally in the Indian snake root plant *Rauwolfia,* was widely used to treat mental illness and was one of the first genuinely effective psychiatric medicines. In 1960, after four years of work, Woodward synthesized **chlorophyll**, the light energy capturing pigment in green plants, and in 1962 he accomplished the total synthesis of a tetracycline antibiotic.

Total synthesis requires the design and then precise implementation of elaborate procedures composed of many steps. Each step in a synthetic procedure either adds or subtracts chemical groups from a starting molecule or rearranges the orientation or order of the atoms in the molecule. Since it is impossible, even with the utmost care, to achieve one hundred percent conversion of starting compound to product at any given step, the greater the number of steps, the less product is obtained.

Woodward and Doering produced approximately a half a gram of quinine from about five pounds of starting materials; they began with benzaldehyde, a simple, inexpensive chemical obtained from coal tar, and designed a 17-step synthetic procedure. The 20-step synthesis that led to the first steroid **nucleus** required 22 lb (10 kg) of starting material and yielded less than a twentieth of an ounce of product. The best synthesis schemes thus have the fewest number of steps, although for some very complicated molecules, "few" may mean several dozen. When Woodward successfully synthesized chlorophyll (which has an elaborate interconnected ring structure), for example, he required 55 steps for the synthesis.

Woodward's close friend, Nobel Laureate Vladimir Prelog, helped establish the CIBA-Geigy Corporation-funded Woodward Institute in Zurich, Switzerland, in the early 1960s. There, Woodward could work on whatever project he chose, without the intrusion of teaching or administrative duties. Initially, the Swiss Federal Institute of Technology had tried to hire Woodward away from Harvard; when it failed, the Woodward Institute provided an alternative way of ensuring that Woodward visited and worked frequently in Switzerland. In 1965, Woodward and his Swiss collaborators synthesized Cephalosporin C, an important antibiotic. In 1971 he succeeded in synthesizing vitamin B_{12}, a molecule bearing some chemical similarity to chlorophyll, but with cobalt instead of magnesium as the central metal atom. Until the end of his life, Woodward worked on the synthesis of the antibiotic erythromycin.

Woodward, who received a Nobel Prize in 1965, helped start two organic chemistry journals, *Tetrahedron Letters* and *Tetrahedron,* served on the boards of several science organizations, and received awards and honorary degrees from many countries. Some of his many honors include the Davy Medal (1959) and the Copley Medal (1978), both from the Royal Society of Britain, and the United States' National Medal of Science (1964). He reached full professor status at Harvard in 1950 and in 1960 became the Donner Professor of Science. Woodward supervised more than three hundred graduate students and postdoctoral students throughout his career.

Woodward married Irji Pullman in 1938 and had two daughters. He was married for the second time in 1946 to Eudoxia Muller, who had also been a consultant at the Polaroid Corporation. The couple had two children. Woodward died at his home of a heart attack on July 8, 1979, at the age of 62.

See also Biochemical analysis techniques; Biochemistry; History of the development of antibiotics

World Health Organization (WHO)

The World Health Organization (WHO) is the principle international organization managing **public health** related issues on a global scale. Headquartered in Geneva, the WHO is comprised of 191 member states (e.g., countries) from around the globe. The organization contributes to international public health in areas including disease prevention and control, promotion of good health, addressing diseases outbreaks, initiatives to eliminate diseases (e.g., **vaccination** programs), and development of treatment and prevention standards.

The genesis of the WHO was in 1919. Then, just after the end of World War I, the League of Nations was created to promote peace and security in the aftermath of the war. One of the mandates of the League of Nations was the prevention and control of disease around the world. The Health Organization of the League of Nations was established for this purpose, and was headquartered in Geneva. In 1945, the United Nations Conference on International Organization in San Francisco approved a motion put forth by Brazil and China to establish a new and independent international organization devoted to public health. The proposed organization was meant to unite the number of disparate health organizations that had been established in various countries around the world.

The following year this resolution was formally enacted at the International Health Conference in New York, and the Constitution of the World Health organization was approved. The Constitution came into force on April 7, 1948. The first Director General of WHO was Dr. Brock Chisholm, a psychiatrist from Canada. Chisholm's influence was evident in the Constitution, which defines health as not merely the absence of disease. A definition that subsequently paved the way for WHO's involvement in the preventative aspects of disease.

From its inception, WHO has been involved in public health campaigns that focus on the improvement of sanitary conditions. In 1951, the Fourth World Health Assembly adopted a WHO document proposing new international sanitary regulations. Additionally, WHO mounted extensive vaccination campaigns against a number of diseases of microbial origin, including **poliomyelitis**, **measles**, **diphtheria**, whooping cough, **tetanus**, **tuberculosis**, and **smallpox**. The latter campaign has been extremely successful, with the last known natural case of smallpox having occurred in 1977. The elimination of poliomyelitis is expected by the end of the first decade of the twenty-first century.

Another noteworthy initiative of WHO has been the Global Programme on **AIDS**, which was launched in 1987. The participation of WHO and agencies such as the **Centers for Disease Control** and Prevention is necessary to adequately address AIDS, because the disease is prevalent in under-developed countries where access to medical care and health promotion is limited.

Today, WHO is structured as eight divisions. The themes that are addressed by individual divisions include communicable diseases, noncommunicable diseases and mental health, family and community health, sustainable development and health environments, health technology and pharmaceuticals, and policy development. These divisions support the four pillars of WHO: worldwide guidance in health, worldwide development of improved standards of health, cooperation with governments in strengthening national health programs, and development of improved health technologies, information, and standards.

See also History of public heath; Public health, current issues

WRIGHT, ALMROTH EDWARD (1861-1947)

English bacteriologist and immunologist

Almroth Edward Wright is best known for his contributions to the field of **immunology** and the development of the autogenous **vaccine**. Wright utilized **bacteria** that were present in the host to create his vaccines. He also developed an anti-typhoid inoculation composed of heat-killed **typhus** specific bacilli. Wright was a consistent advocate for vaccine and inoculation therapies, and at the onset of World War I convinced the British military to inoculate all troops against typhus. However, Wright was also interested in bacteriological research. Wright conducted several studies on bacteriological infections in post-surgical and accidental wounds.

Wright was born in Yorkshire, England. He studied medicine at Trinity College Dublin, graduating in 1884. He then studied medicine in France, Germany, and Australia for few years before returning home to accept a position in London. He conducted most of his research at the Royal Victoria Hospital where he was Chair of Pathology at the Army Medical School. In 1899, Wright lobbied to have all of the troops departing to fight in the Boer War in Africa inoculated against typhus. The government permitted Wright to institute a voluntary program, but only a small fraction of troops participated. Typhus was endemic among the soldiers in Africa, and accounted for over 9,000 deaths during the war. Following the return of the troops, the Army conducted a study into the efficacy of the inoculation and for unknown reasons, decided to suspend the inoculation program. Wright was infuriated and resigned his post.

Wright then took a position at St. Mary's Hospital in London. He began a small **vaccination** and inoculation clinic that later became the renowned Inoculation Department. Convinced that his anti-typhus inoculation worked, he arranged for a second study of his therapy on British troops stationed in India. The results were promising, but the Army largely ignored the new information. Before the eve of World War I, Wright once again appealed to military command to inoculate troops against typhus. Wright petitioned Lord Kitchener in 1914. Kitchener agreed with Wright's recommendation and ordered a mandatory inoculation program.

Most likely owing to his often sparse laboratory settings, Wright revised several experimental methods, publishing them in various journals. One of his most renowned contributions was a reform of common blood and fluid collection procedures. Common practice was to collect samples from capillaries with pipettes, not from veins with a syringe. Like modern syringes, pipettes required suction. This was usually supplied by mouth. Wright attached a rubberized teat to the **pipette**, permitting for a cleaner, more aseptic, collection of blood and fluid samples. He also developed a disposable capsule for the collection, testing, and storage of blood specimens. In 1912, Wright published a compendium of several of his reformed techniques.

Wright often had to endure the trials of critical colleagues and **public health** officials who disagreed with some of his innovations in the laboratory and his insistence on vaccine therapies. Wright usually prevailed in these clashes. However, Wright stood in opposition to the most formidable medical movement of his early days, antisepsis. Antiseptic surgical protocols called for the **sterilization** of all instruments and surgical surfaces with a carbolic acid solution. However, some surgeons and proponents of the practice advocated placing bandages soaked in a weaker form of the solution directly on patient wounds. Wright agreed with the practice of instrument sterilization, but claimed that antiseptic wound care killed more leukocytes, the body's natural defense against bacteria and infection, than harmful bacteria. Wright's solution was to treat wounds with a saline wash and let the body fight infection with its own defenses. Not until the advancement of asepsis, the process of creating a sterile environment within the hospital, and the discovery of **antibiotics** was Wright's claim re-evaluated.

Wright had a distinguished career in his own right, but is also remembered as the teacher of **Alexander Fleming**, who later discovered **penicillin** and antibiotics. During Wright's campaign to inoculate troops before World War I, and throughout the course of his research on wound care, Fleming was Wright's student and assistant. Fleming's later research vindicated many of Wright's theories on wound care, but also lessened the significance of autogenous vaccine therapies. The Inoculation Department in which both Wright and Fleming worked was later renamed in honor of the two scientists.

Wright died, while still actively working at his laboratory in Buckinghamshire, at the age of 85.

See also Immune stimulation, as a vaccine; Immune system; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunization

X

XANTHOPHYLLS

Photosynthesis is the conversion of light energy into chemical energy utilized by plants, many algae, and cyanobacteria. However, each photosynthetic organism must be able to dissipate the light radiation that exceeds its capacity for carbon dioxide fixation before it can damage the photosynthetic apparatus (i.e., the **chloroplast**). This photoprotection is usually mediated by oxygenated carotenoids, i.e., a group of yellow pigments termed xanthophylls, including violaxanthin, antheraxanthin, and zeaxanthin, which dissipate the thermal radiation from the sunlight through the xanthophyll cycle.

Xanthophylls are present in two large protein-cofactor complexes, present in photosynthetic membranes of organisms using Photosystem I or Photosystem II. Photosystem II uses water as electron donors, and pigments and quinones as electron acceptors, whereas the Photosystem I uses plastocyanin as electron donors and iron-sulphur centers as electron acceptors. Photosystem I in thermophilic Cyanobacteria, for instance, is a crystal structure that contains 12 protein subunits, 2 phylloquinones, 22 carotenoids, 127 cofactors constituting 96 chlorophylls, besides calcium cations, **phospholipids**, three iron-sulphur groups, water, and other elements. This apparatus captures light and transfers electrons to pigments and at the same time dissipates the excessive excitation energy via the xanthophylls.

Xanthophylls are synthesized inside the plastids and do not depend on light for their synthesis as do chlorophylls. From dawn to sunset, plants and other photosynthetic organisms are exposed to different amounts of solar radiation, which determine the xanthophyll cycle. At dawn, a pool of diepoxides termed violaxanthin is found in the plastids, which will be converted by the monoepoxide antheraxanthin into zeaxanthin as the light intensity gradually increases during the day. Zeaxanthin absorbs and dissipates the excessive solar radiation that is not used by **chlorophyll** during carbon dioxide fixation. At the peak hours of sunlight exposition, almost all xanthophyll in the pool is found under the form of zeaxanthin, which will be gradually reconverted into violaxanthin as the solar radiation decreases in the afternoon to be reused again in the next day.

See also Autotrophic bacteria; Photosynthetic microorganisms

XANTHOPHYTA

The yellow-green algae are photosynthetic species of organisms belonging to the Xanthophyta Phylum, which is one of the phyla pertaining to the Chromista Group in the Protista Kingdom. Xanthophyta encompasses 650 living species so far identified. Xanthophyta live mostly in freshwater, although some species live in marine water, tree trunks, and damp soils. Some species are unicellular organisms equipped with two unequal flagella that live as free-swimming individuals, but most species are filamentous. Filamentous species may be either siphonous or coenocytic. Coenocytes are organized as a single-cell multinucleated thallus that form long filaments without septa (internal division walls) except in the specialized structures of some species. Siphonous species have multiple tubular cells containing several nuclei.

Xanthophyta synthesize **chlorophyll** a and smaller amounts of chlorophyll c, instead of the chlorophyll b of plants; and the cellular structure usually have multiple chloroplasts without nucleomorphs. The plastids have four membranes and their yellow-green color is due to the presence of beta-carotene and xanthins, such as vaucheriaxanthin, diatoxanthin, diadinoxanthin, and heretoxanthin, but not fucoxanthin, the brown pigment present in other Chromista. Because of the presence of significant amounts of chlorophyll a, Xanthophyceae species are easily mistaken for green algae. They store polysaccharide under the form of chrysolaminarin and carbohydrates as oil droplets.

One example of a relatively common Xanthophyta is the class Vaucheria that gathers approximately 70 species, whose structure consists of several tubular filaments, sharing

its nuclei and chloroplasts without septa. They live mainly in freshwater, although some species are found in seawater spreading along the bottom like a carpet. Other Xanthophyceae Classes are Tribonema, whose structure consists of unbranched filaments; Botrydiopsis, such as the species *Botrydium* with several thalli, each thallus formed by a large aerial vesicle and rhizoidal filaments, found in damp soil; Olisthodiscus, such as the species *Ophiocytium* with cylindrical and elongated multinucleated cells and multiple chloroplasts.

See also Photosynthetic microorganisms; Protists

Y

YALOW, ROSALYN SUSSMAN (1921-)

American medical physicist

Rosalyn Sussman Yalow was co-developer of radioimmunoassay (RIA), a technique that uses radioactive isotopes to measure small amounts of biological substances. In widespread use, the RIA helps scientists and medical professionals measure the concentrations of hormones, vitamins, **viruses**, **enzymes**, and drugs, among other substances. Yalow's work concerning RIA earned her a share of the Nobel Prize in physiology or medicine in the late 1970s. At that time, she was only the second woman to receive the Nobel Prize in medicine. During her career, Yalow also received acclaim for being the first woman to attain a number of other scientific achievements.

Yalow was born on July 19, 1921, in The Bronx, New York, to Simon Sussman and Clara Zipper Sussman. Her father, owner of a small business, had been born on the Lower East Side of New York City to Russian immigrant parents. At the age of four, Yalow's mother had journeyed to the United States from Germany. Although neither parent had attended high school, they instilled a great enthusiasm for and respect of education in their daughter. Yalow also credits her father with helping her find the confidence to succeed in school, teaching her that girls could do just as much as boys. Yalow learned to read before she entered kindergarten, although her family did not own many books. Instead, Yalow and her older brother, Alexander, made frequent visits to the public library.

During her youth, Yalow became interested in mathematics. At Walton High School in the Bronx, her interest turned to science, especially chemistry. After graduation, Yalow attended Hunter College, a women's school in New York that eventually became part of the City University of New York. She credits two physics professors, Dr. Herbert Otis and Dr. Duane Roller, for igniting her penchant for physics. This occurred in the latter part of the 1930s, a time when many new discoveries were made in nuclear physics. It was this field that Yalow ultimately chose for her major. In 1939, she was further inspired after hearing American physicist Enrico Fermi lecture about the discovery of nuclear fission, which had earned him the Nobel Prize the previous year.

As Yalow prepared for her graduation from Hunter College, she found that some practical considerations intruded on her passion for physics. In fact, Yalow's parents urged her to pursue a career as an elementary school teacher. Yalow herself also thought it unrealistic to expect any of the top graduate schools in the country to accept her into a doctoral program or offer her the financial support that men received. "However, my physics professors encouraged me and I persisted," she explained in *Les Prix Nobel 1977*.

Yalow made plans to enter graduate school via other means. One of her earlier college physics professors, who had left Hunter to join the faculty at the Massachusetts Institute of Technology, arranged for Yalow to work as secretary to Dr. Rudolf Schoenheimer, a biochemist at Columbia University in New York. According to the plan, this position would give Yalow an opportunity to take some graduate courses in physics, and eventually provide a way for her to enter a graduate a school and pursue a degree. But Yalow never needed her plan. The month after graduating from Hunter College in January 1941, she was offered a teaching assistantship in the physics department of the University of Illinois at Champaign-Urbana.

Gaining acceptance to the physics graduate program in the College of Engineering at the University of Illinois was one of many hurdles that Yalow had to cross as a woman in the field of science. For example, when she entered the University in September 1941, she was the only woman in the College of Engineering's faculty, which included 400 professors and teaching assistants. She was the first woman in more than two decades to attend the engineering college. Yalow realized that she had been given a space at the prestigious graduate school because of the shortage of male candidates, who were being drafted into the armed services in increasing numbers as America prepared to enter World War II.

Yalow's strong work orientation aided her greatly in her first year in graduate school. In addition to her regular course

load and teaching duties, she took some extra undergraduate courses to increase her knowledge. While in graduate school she also met Aaron Yalow, a fellow student and the man she would eventually marry. The pair met the first day of school and wed about two years later on June 6, 1943. Yalow received her master's degree in 1942 and her doctorate in 1945. She was the second woman to obtain a Ph.D. in physics at the University.

After graduation the Yalows moved to New York City, where they worked and eventually raised two children, Benjamin and Elanna. Yalow's first job after graduate school was as an assistant electrical engineer at Federal Telecommunications Laboratory, a private research lab. Once again, she found herself the sole woman as there were no other female engineers at the lab. In 1946, she began teaching physics at Hunter College. She remained a physics lecturer from 1946 to 1950, although by 1947, she began her long association with the Veterans Administration by becoming a consultant to Bronx VA Hospital. The VA wanted to establish some research programs to explore medical uses of radioactive substances. By 1950, Yalow had equipped a radioisotope laboratory at the Bronx VA Hospital and decided to leave teaching to devote her attention to full-time research.

That same year, Yalow met Solomon A. Berson, a physician who had just finished his residency in internal medicine at the hospital. The two would work together until Berson's death in 1972. According to Yalow, the collaboration was a complementary one. In Olga Opfell's *Lady Laureates,* Yalow is quoted as saying, "[Berson] wanted to be a physicist, and I wanted to be a medical doctor." While her partner had accumulated clinical expertise, Yalow maintained strengths in physics, math, and chemistry. Working together, Yalow and Berson discovered new ways to use radioactive isotopes in the measurement of blood volume, the study of iodine **metabolism**, and the diagnosis of thyroid diseases. Within a few years, the pair began to investigate adult-onset diabetes using radioisotopes. This project eventually led them to develop the groundbreaking radioimmunoassay technique.

In the 1950s, some scientists hypothesized that in adult-onset diabetes, insulin production remained normal, but a liver enzyme rapidly destroyed the peptide hormone, thereby preventing normal glucose metabolism. This contrasted with the situation in juvenile diabetes, where insulin production by the pancreas was too low to allow proper metabolism of glucose. Yalow and Berson wanted to test the hypothesis about adult-onset diabetes. They used insulin "labeled" with ^{131}iodine (that is, they attached, by a chemical reaction, the radioactive isotope of iodine to otherwise normal insulin molecules.) Yalow and Berson injected labeled insulin into diabetic and non-diabetic individuals and measured the rate at which the insulin disappeared.

To their surprise and in contradiction to the liver enzyme hypothesis, they found that the amount of radioactively labeled insulin in the blood of diabetics was higher than that found in the control subjects who had never received insulin injections before. As Yalow and Berson looked into this finding further, they deduced that diabetics were forming antibodies to the animal insulin used to control their disease. These antibodies were binding to radiolabeled insulin, preventing it from entering cells where it was used in sugar metabolism. Individuals who had never taken insulin before did not have these antibodies and so the radiolabeled insulin was consumed more quickly.

Yalow and Berson's proposal that animal insulin could spur **antibody formation** was not readily accepted by immunologists in the mid–1950s. At the time, most immunologists did not believe that antibodies would form to molecules as small as the insulin peptide. Also, the amount of insulin antibodies was too low to be detected by conventional immunological techniques. Yalow and Berson set out to verify these minute levels of insulin antibodies using radiolabeled insulin as their marker. Their original report about insulin antibodies, however, was rejected initially by two journals. Finally, a compromise version was published that omitted "insulin antibody" from the paper's title and included some additional data indicating that an **antibody** was involved.

The need to detect insulin antibodies at low concentrations led to the development of the radioimmunoassay. The principle behind RIA is that a radiolabeled **antigen**, such as insulin, will compete with unlabeled antigen for the available binding sites on its specific antibody. As a standard, various mixtures of known amounts of labeled and unlabeled antigen are mixed with antibody. The amounts of radiation detected in each sample correspond to the amount of unlabeled antigen taking up antibody binding sites. In the unknown sample, a known amount of radiolabeled antigen is added and the amount of radioactivity is measured again. The radiation level in the unknown sample is compared to the standard samples; the amount of unlabeled antigen in the unknown sample will be the same as the amount of unlabeled antigen found in the standard sample that yields the same amount of radioactivity. RIA has turned out to be so useful because it can quickly and precisely detect very low concentrations of hormones and other substances in blood or other biological fluids. The principle can also be applied to binding interactions other than that between antigen and antibody, such as between a binding protein or tissue receptor site and an enzyme. In Yalow's Nobel lecture, recorded in *Les Prix Nobel 1977,* she listed more than 100 biological substances—hormones, drugs, vitamins, enzymes, viruses, non-hormonal proteins, and more—that were being measured using RIA.

In 1968, Yalow became a research professor at the Mt. Sinai School of Medicine, and in 1970, she was made chief of the Nuclear Medicine Service at the VA hospital. Yalow also began to receive a number of prestigious awards in recognition of her role in the development of RIA. In 1976, she was awarded the Albert Lasker Prize for Basic Medical Research. She was the first woman to be honored this laurel—an award that often leads to a Nobel Prize. In Yalow's case, this was true, for the very next year, she shared the Nobel Prize in physiology or medicine with Andrew V. Schally and Roger Guillemin for their work on radioimmunoassay. Schally and Guillemin were recognized for their use of RIA to make important discoveries about brain hormones.

Berson had died in 1972, and so did not share in these awards. According to an essay in The Lady Laureates, she

remarked that the "tragedy" of winning the Nobel Prize "is that Dr. Berson did not live to share it." Earlier Yalow had paid tribute to her collaborator by asking the VA to name the laboratory, in which the two had worked, the Solomon A. Berson Research Laboratory. She made the request, as quoted in *Les Prix Nobel 1977,* "so that his name will continue to be on my papers as long as I publish and so that his contributions to our Service will be memorialized."

Yalow has received many other awards, honorary degrees, and lectureships, including the Georg Charles de Henesy Nuclear Medicine Pioneer Award in 1986 and the Scientific Achievement Award of the American Medical Society. In 1978, she hosted a five-part dramatic series on the life of French physical chemist Marie Curie, aired by the Public Broadcasting Service (PBS). In 1980, she became a distinguished professor at the Albert Einstein College of Medicine at Yeshiva University, leaving to become the Solomon A. Berson Distinguished Professor at Large at Mt. Sinai in 1986. She also chaired the Department of Clinical Science at Montefiore Hospital and Medical Center in the early- to mid-1980s.

The fact that Yalow was a trailblazer for women scientists was not lost on her. At a lecture before the Association of American Medical Colleges, as quoted in *Lady Laureates,* Yalow opined: "We cannot expect that in the foreseeable future women will achieve status in academic medicine in proportion to their numbers. But if we are to start working towards that goal we must believe in ourselves or no one else will believe in us; we must match our aspirations with the guts and determination to succeed; and for those of us who have had the good fortune to move upward, we must feel a personal responsibility to serve as role models and advisors to ease the path for those who come afterwards."

See also Laboratory techniques in immunology; Radioisotopes and their uses in microbiology and immunology

YEAST

Yeasts are single-celled **fungi**. Yeast species inhabit diverse habitats, including skin, marine water, leaves, and flowers.

Some yeast are beneficial, being used to produce bread or allow the **fermentation** of sugars to ethanol that occurs during beer and wine production (e.g., ***Saccharomyces cerevisiae***). Other species of yeasts are detrimental to human health. An example is *Candida albicans*, the cause of vaginal infections, diaper rash in infants, and **thrush** in the mouth and throat. The latter infection is fairly common in those whose **immune system** is compromised by another infection such as acquired **immunodeficiency** syndrome.

The economic benefits of yeast have been known for centuries. *Saccharomyces carlsbergensis*, the yeast used in the production of various types of beer that result from "bottom fermentation," was isolated in 1888 by Dr. Christian Hansen at the Carlsberg Brewery in Copenhagen. During fermentation, some species of yeast are active at the top of the brew while others sink to the bottom. In contrast to *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae* produces ales by "top fermentation." In many cases, the genetic manipulation of yeast has eliminated the need for the different yeast strains to produce beer or ale. In baking, the fermentation of sugars by the bread yeast *Ascomycetes* produces bubbles in the dough that makes the bread dough rise.

Yeasts are a source of B vitamins. This can be advantageous in diets that are low in meat. In the era of **molecular biology**, yeasts have proved to be extremely useful research tools. In particular, *Saccharomyces cerevisiae* has been a model system for studies of genetic regulation of cell division, **metabolism**, and the incorporation of genetic material between organisms. This is because the underlying molecular mechanisms are preserved in more complicated **eukaryotes**, including humans, and because the yeast cells are so easy to grow and manipulate. As well, *Ascomycetes* are popular for genetics research because the genetic information contained in the spores they produce result from meiosis. Thus, the four spores that are produced can contain different combinations of genetic material. This makes the study of genetic inheritance easy to do.

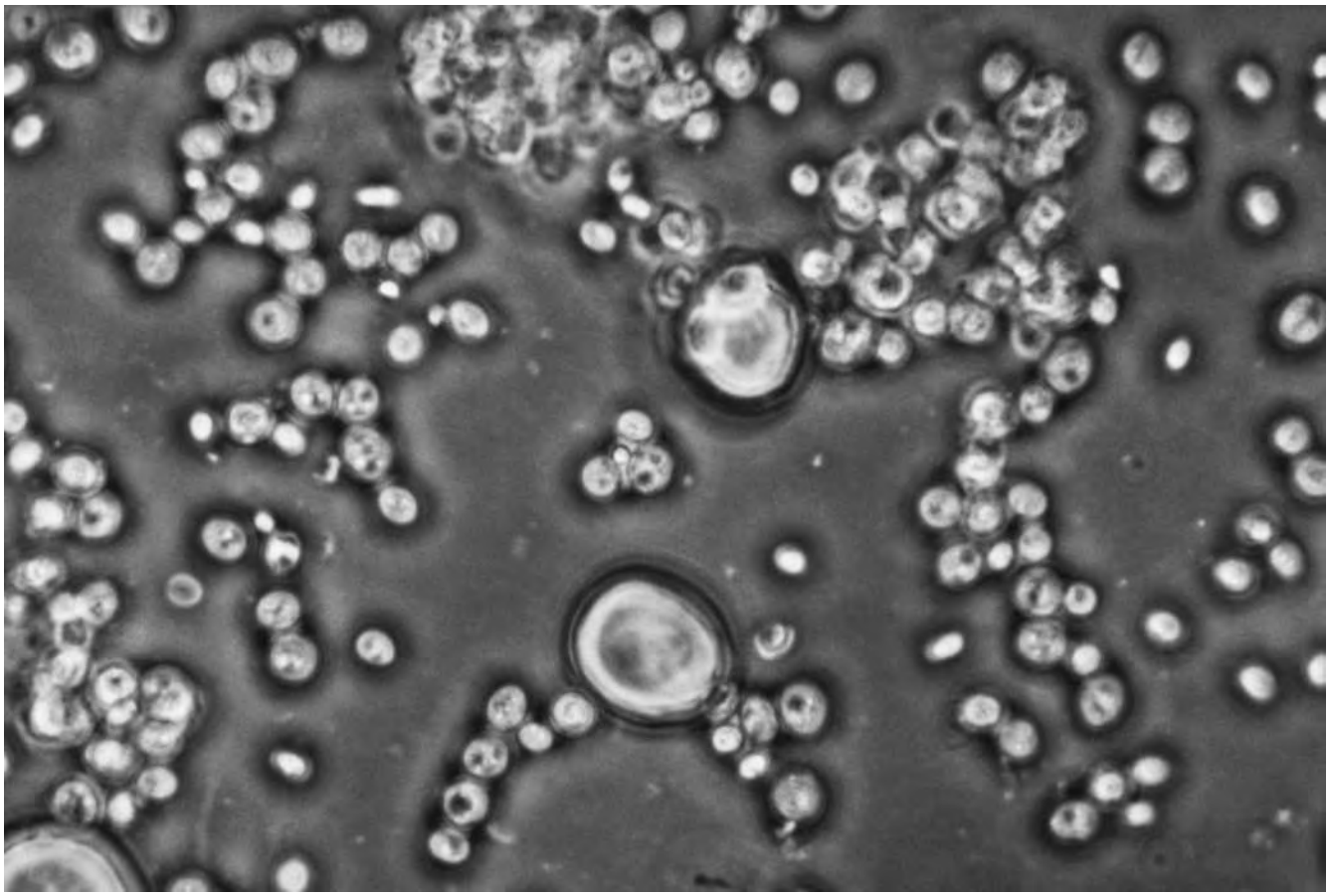
Another feature of yeast that makes them attractive as models of study is the ease by which their genetic state can be manipulated. At different times in the **cell cycle** yeast cells will contain one copy of the genetic material, while at other times two copies will be present. Conditions can be selected that maintain either the single or double-copy state. Furthermore, a myriad of yeast **mutants** have been isolated or created that are defective in various aspects of the cell division cycle. These mutants have allowed the division cycle to be deduced in great detail.

The division process in yeast occurs in several different ways, depending upon the species. Some yeast cells multiply by the formation of a small bud that grows to be the size of the parent cell. This process is referred to as budding. *Saccharomyces* reproduces by budding. The budding process is a sexual process, meaning that the genetic material of two yeast cells is combined in the offspring. The division process involves the formation of spores.

Other yeasts divide by duplicating all the cellular components and then splitting into two new daughter cells. This process, called binary fission, is akin to the division process in **bacteria**. The yeast genus *Schizosaccharomyces* replicates in this manner. This strain of yeast is used as a teaching tool because the division process is so easy to observe using an inexpensive light **microscope**.

The growth behavior of yeast is also similar to bacteria. Yeast cells display a lag phase prior to an explosive period of division. As some nutrient becomes depleted, the increase in cell number slows and then stops. If refrigerated in this stationary phase, cells can remain alive for months. Also like bacteria, yeast are capable of growth in the presence and the absence of oxygen.

The life cycle of yeast includes a step called meiosis. In meiosis pairs of **chromosomes** separate and the new combinations that form can give rise to new genetic traits in the daughter yeast cells. Meiosis is also a sexual feature of genetic replication that is common to all higher eukaryotes as well.



Light micrograph of baker's yeast.

Another feature of the sexual reproduction process in yeast is the production of pheromones by the cells. Yeast cells respond to the presence of the chemicals by changing their shape. The peanut-like shape they adopt has been dubbed "shmoos," after a character in the "Li'l Abner" comic strip. This shape allows two cells to associate very closely together.

See also Cell cycle (eukaryotic), genetic regulation of; Chromosomes, eukaryotic; Economic uses and benefits of microorganisms; Yeast artificial chromosome; Yeast, infectious

YEAST ARTIFICIAL CHROMOSOME (YAC)

The **yeast** artificial chromosome, which is often shortened to YAC, is an artificially constructed system that can undergo replication. The design of a YAC allows extremely large segments of genetic material to be inserted. Subsequent rounds of replication produce many copies of the inserted sequence, in a genetic procedure known as **cloning**.

The reason the cloning vector is called a yeast artificial chromosome has to do with the structure of the vector. The YAC is constructed using specific regions of the yeast chromosome. Yeast cells contain a number of **chromosomes**; organized collections of **deoxyribonucleic acid** (**DNA**). For example, the yeast *Saccharomyces cerevisae* contains 16 chromosomes that contain varying amounts of DNA. Each chromosome consists of two arms of DNA that are linked by a region known as the centromere. As the DNA in each arm is duplicated, the centromere provides a region of common linkage. This common area is the region to which components of the replication machinery of the cell attach and pull apart the chromosomes during the cell division process. Another region of importance is called the telomere. The end of each chromosome arm contains a region of DNA called the telomere. The telomere DNA does not code for any product, but serves as a border to define the size of the chromosome. Finally, each chromosome contains a region known as the origin of replication. The origin is where a molecule called DNA polymerase binds and begins to produce a copy of each strand of DNA in the double helix that makes up the chromosome.

The YAC was devised and first reported in 1987 by David Burke, who then also reported the potential to use the construct as a cloning vehicle for large pieces of DNA. Almost immediately, YACs were used in large-scale determi-

nation of genetic sequences, most prominently the Human Genome Project.

YAC contains the telomere, centromere, and origin of replication elements. If these elements are spliced into DNA in the proper location and orientation, then a yeast cell will replicate the artificial chromosome along with the other, natural chromosomes. The target DNA is flanked by the telomere regions that mark the ends of the chromosome, and is interspersed with the centromere region that is vital for replication. Finally, the start site for the copying process is present. In essence, the yeast is fooled into accepted genetic material that mimics a chromosome.

The origin of the DNA that is incorporated into a YAC is varied. DNA from prokaryotic organisms such as bacterial or from **eukaryotes** such a humans can be successfully used. The power of YACs is best explained by the size of the DNA that can be copied. **Bacteria** are also capable of cloning DNA from diverse sources, but the length of DNA that a bacterium can handle is up to 20 times less than that capable of being cloned using a YAC.

The engineered YAC is put back into a yeast cell by chemical means that encourage the cell to take up the genetic material. As the yeast cell undergoes rounds of growth and division, the artificial chromosome is replicated as if it were a natural chromosomal constituent of the cell. The result is a **colony** of many genetically identical yeast cells, each containing a copy of the target DNA. The target DNA has thus been amplified in content. Through a subsequent series of procedures, DNA can then be isolated from the rest of the DNA inside the yeast cells.

Use of different regions of DNA in different YACs allows the rapid determination of the sequence, or order of the constituents, of the DNA. YACs were invaluable in this regard in the sequencing of the human genome, which was completed in preliminary form in 2001 The human genome was broken into pieces using various **enzymes**. Each piece could be used to construct a YAC. Then, sufficient copies of each piece of the human genome could be generated so that automatic sequencing machines would have enough material to sequence the DNA.

Commonly, the cutting enzymes are selected so that the fragments of DNA that are generated contain overlapping regions. Once the sequences of all the DNA regions are obtained the common overlapping regions allow the fragment sequences to be chemically bonded so that the proper order and the proper orientation is generated. For example, if no overlapping regions were present, then one sequence could be inserted backwards with respect to the orientation of its neighbouring sequence.

See also Chromosomes, prokaryotic; Gene amplification; Yeast genetics

YEAST, ECONOMIC USES AND BENEFITS ·

see ECONOMIC USES AND BENEFITS OF MICROORGANISMS

YEAST GENETICS

Yeast genetics provides an excellent model for the study of the genetics of growth in animal and plant cells. The yeast *Saccharomyces cerevisiae* is similar to animal cells (e.g., similar length to the phases of its **cell cycle**, similarity of the chromosomal structures called telomeres). Another yeast, *Saccharomyces pombe* is rather more similar to plant cells (e.g., similarities in their patterns of division, and in organization of their genome).

As well as being a good model system to study the mechanics of eukaryotic cells, yeast is well suited for genetic studies. Yeasts are easy to work with in the laboratory. They have a rapid growth cycle (1.5 to two hours), so that many cycles can be studied in a day. Yeasts that are not a health threat are available, so the researcher is usually not in danger when handling the organisms. Yeasts exist that can be maintained with two copies of their genetic material (diploid state) or one copy (haploid state). Haploid strains can be mated together to produce a diploid that has genetic traits of both "parents." Finally, it is easy to introduce new **DNA** sequences into the yeast.

Genetic studies of the yeast cell cycle, the cycle of growth and reproduction, are particularly valuable. For example, the origin of a variety of cancers is a malfunction in some aspect of the cell cycle. Various strains of ***Saccharomyces cerevisiae*** and *Saccharomyces pombe* provide useful models of study because they are also defective in some part of their cell division cycle. In particular, cell division cycle (cdc) **mutants** are detected when the point in the cell cycle is reached where the particular protein coded for by the defective **gene** is active. These points where the function of the protein is critical have been dubbed the "execution points." **Mutations** that affect the cell division cycle tend to be clustered at two points in the cycle. One point is at the end of a phase known as G1. At the end of G1 a yeast cell becomes committed to the manufacture of DNA in the next phase of the cell cycle (S phase). The second cluster of mutations occurs at the beginning of a phase called the M phase, where the yeast cell commits to the separation of the chromosomal material in the process of mitosis.

Lee Hartwell of the University of Washington at Seattle spearheaded the analysis of the various cdc mutants in the 1960s and 1970s. His detailed examination of the blockage of the cell cycle at certain points—and the consequences of the blocks on later events—demonstrated, for example, that the manufacture of DNA was an absolute prerequisite for division of the nuclear material. In contrast the formation of the bud structures by *Saccharomyces pombe* can occur even when DNA replication is blocked.

Hartwell also demonstrated that the cell cycle depends on the completion of a step that was termed "start." This step is now known to be a central control point, where the cell essentially senses materials available to determine whether the growth rate of the cell will be sufficient to accumulate enough material to permit cell division to occur. Depending on the information, a yeast cell either commits to another cycle of cell growth and division or does not. These events have been

confirmed by the analysis of a yeast cell mutant called cdc28. The cdc28 mutant is blocked at start and so does not enter S phase where the synthesis of DNA occurs.

Analysis of this and other cdc mutations has found a myriad of functions associated with the genetic mutations. For example, in yeast cells defective in a gene dubbed cdc2, the protein coded for by the cdc2 gene does not modify various proteins. The absence of these modifications causes defects in the aggregation of the chromosomal material prior to mitosis, the change in the supporting structures of the cell that are necessary for cell division, and the ability of the cell to change shape.

Studies of such cdc mutants has shown that virtually all eukaryotic cells contain a similar control mechanism that governs the ability of a cell to initiate mitosis. This central control point is affected by the activities of other proteins in the cell. A great deal of research effort is devoted to understanding this master control, because scientists presume that knowledge of its operation could help thwart the development of cancers related to a defect in the master control.

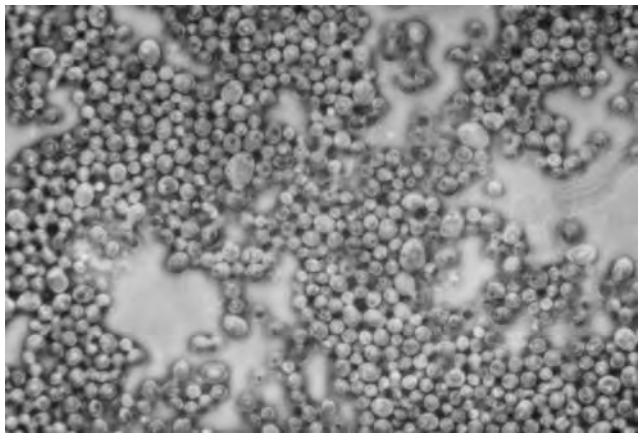
See also Cell cycle (eukaryotic), genetic regulation of; Genetic regulation of eukaryotic cells; Molecular biology and molecular genetics

YEAST, INFECTIOUS

Yeast are single-cell **fungi** with ovoid or spherical shapes, which are grouped according to the cell division process into budding yeast (e.g., the species and strains of ***Saccharomyces cerevisiae*** and *Blastomyces dermatitidis*), or fission yeast (e.g., *Schizosaccharomyces*) species.

Yeast species are present in virtually all natural environments such as fresh and marine water, soil, plants, animals, and in houses, hospitals, schools, etc. Some species are symbiotic, while others are parasitic. Parasitic species may be pathogenic (i.e., cause disease) either because of the toxins they release in the host organism or due to the direct destruction of living tissues such as skin, internal mucosa of the mouth, lungs, gastrointestinal, genital and urinary tracts of animals, along with plant flowers, fruits, seeds, and leaves. They are also involved in the deterioration and **contamination** of stored grains and processed foods.

Yeast and other fungal infections may be superficial (skin, hair, nails); subcutaneous (dermis and surrounding structures); systemic (affecting several internal organs, blood, and internal epithelia); or opportunistic (infecting neutropenic patients, such as cancer patients, transplant patients, and other immunocompromised patients). Opportunistic infections acquired by patients inside hospitals, or due to medical procedures such as catheters are termed **nosocomial infections**, and they are a major concern in **public health**, because they increase both mortality and the period of hospitalization. An epidemiological study, with data collected between 1997 and 2001 in 72 different hospitals in the United States, showed that 7–8% of the nosocomial blood-stream infections were due to a *Candida* species of yeast, especially *Candida albicans*. About 80% of *Candida* infections are nosocomial in the United States, and approximately 50% of them are acquired in intensive care units. A national **epidemiology** of mycoses survey in the early 1990s showed that in neonatal ICUs *C. albicans* was the cause of about 75% of infections and *Candida parapsilosis* accounted for the remaining 25%. *Candida albicans* frequently infects infants during birth, due to its presence in the mother's vaginal mucosa, whereas *C. parapsilosis* was found in the hands of healthcare professionals of the neonatal ICUs. In surgical ICUs, *C. albicans* was implicated in 50% of infections while *Candida glabrata* responded for another 25% of the cases. The most frequently community-acquired yeast infections are the superficial mycoses, and among other pathogenic fungi, *Candida albicans* is the cause of mouth **thrush**, and vaginitis. Gastrointestinal yeast infections are also transmitted by contaminated saliva and foods.



Light micrograph of *Candida albicans*.

Although immunocompetent individuals may host *Candida* species and remain asymptomatic for many years, the eventual occurrence of a debilitating condition may trigger a systemic **candidiasis**. Systemic candidiasis is a chronic infection that usually starts in the gastrointestinal tract and gradually spreads to other organs and tissues, and the *Candida* species commonly involved is *C. albicans*. They release about 79 different toxins in the hosts' organism, and the lesions they cause in the intestinal membranes compromise nutrient absorption by reducing it to about 50% of the normal capacity. *C. albicans* intestinal colonization and lesions expose internal tissues and capillary vessels to contamination by **bacteria** present in fecal material. The elderly, cancer patients, and infants are especially susceptible to *Candida* infections, as are **AIDS** patients. In the long run, systemic candidiasis may lead to a variety of symptoms, such as chronic fatigue, **allergies**, cystitis, endometriosis, diarrhea, colitis, respiratory disorders, dry mouth, halitosis (bad breath), emotional disorders, etc.

The indiscriminate prescription and intake of **antibiotics** usually kills bacteria that are essential for normal digestion and favors the opportunistic spread of *Candida* species on the walls of the digestive tract, which can be worsened when associated with a diet rich in sugars and carbohydrates. Once yeast species colonize the intestinal walls, treatment becomes difficult and is usually followed by recurrence. Another challenge

when yeast systemic infection is involved is that they are not detected by standard blood tests. However, laboratorial analysis of collected samples of mucus and affected tissue may detect yeast infection and identify the implicated species.

Another yeast infection, known as blastomycosis, is caused by the species *Blastomyces dermatitidis,* a spherical budding yeast. The main targets of this pathogen are the lung alveoli (60%). Pulmonary blastomycosis is not easily diagnosed because its symptoms are also present in other lung infections, such as cough, chest pain, hemoptysis, and weight loss. Pulmonary lesions may include nodules, cavities, and infiltration, with the severe cases presenting pleuritis. Blastomycosis may also be disseminated to other organs, such as liver, central nervous system, adrenal glands, pancreas, bones, lymph nodes, and gastrointestinal and genitourinary tracts. Osteomyelitis (bone infection) and arthritis may also be caused by this yeast, and about 33% of the patients were diagnosed with skeletal blastomycosis as well. Although the cutaneous chronic infection is curable, the systemic form of the disease has a poor prognosis.

See also Food preservation; Food safety; Mycology; Nosocomial infections; Parasites; Yeast artificial chromosome (YAC); Yeast genetics

YELLOW FEVER

Yellow fever is the name given to a disease that is caused by the yellow fever virus. The virus is a member of the flavivirus group. The name of the disease is derived from the appearance of those infected, who usually present a jaundiced appearance (yellow-tinted skin).

The agent of infection of yellow fever is the mosquito. The agent was first identified in 1900 when the United States Army Yellow Fever Commission (also referred to as the Reed Commission after its leader, Walter Reed) proved that the mosquito species *Aedes aegypti* was responsible for spreading the disease. Until then, yellow fever was regarded as requiring direct person-to-person contact or contact with a contaminated object.

The disease has caused large outbreaks involving many people in North America, South America, and Africa, stretching back at least to the 1700s. At that time the disease was often fatal. The availability of a **vaccine** reduced the incidence and mortality of the disease considerably in the latter part of the twentieth century. However, since 1980 the number of cases of the disease has begun to rise again.

There are now about 200,000 estimated cases of yellow fever in the world each year. Of these, some 30,000 people die. Most researchers and health officials regard these numbers as underestimates, due to underreporting and because in the initial stages yellow fever can be misdiagnosed.

The yellow fever virus infects humans and monkeys—no other hosts are known. Humans become infected when the virus is transmitted from monkeys to humans by mosquitoes. This is referred to as horizontal transmission. Several different species of mosquito are capable of transmitting the virus. Mosquitoes can also pass the virus to their own offspring via infected eggs. This form of transmission is called vertical transmission. When the offspring hatch they are already infected and can transmit the virus to humans when they have a blood meal. Vertical transmission can be particularly insidious as the eggs are very hardy and can resist dry conditions, hatching when the next rainy season occurs. Thus the infection can be continued from one year to the next even when there is no active infection occurring in a region.

The different habitats of the mosquitoes ensures a wide distribution of the yellow fever virus. Some of the mosquito species breed in urban areas while others are confined to rural regions. The latter types were associated with the outbreak of yellow fever that struck workers during the construction of the Panama Canal in Central America in the nineteenth century. In South America a concerted campaign to control mosquito populations up until the 1970s greatly reduced the number of cases of yellow fever. However, since that time the control programs have lapsed and yellow fever has increased as the mosquito populations have increased.

Infection with the yellow fever virus sometimes produced no symptoms whatsoever. However, in many people, so-called acute (rapid-onset, intense) symptoms appear about three to six days after infection. The symptoms include fever, muscle pain (particularly in the back), headache, chills, nausea, and vomiting. In this early stage the disease is easily confused with a number of other diseases, including **malaria**, **typhoid fever**, **hemorrhagic fevers** such as Lassa fever, and viral **hepatitis**. Diagnosis requires the detection of an **antibody** to the virus in the blood. Such diagnosis is not always possible in underdeveloped regions or in rural areas that are distant from medical facilities and trained laboratory personnel.

In many people the acute symptoms last only a few days and recovery is complete. However, in about 15% of those infected, the disease enters what is termed the toxic phase: a fever reappears and several regions of the body become infected as the virus disseminates from the point of the mosquito bite. Disruption of liver function produces jaundice. Kidney function can also be damaged and even totally shut down. Recovery from this more serious phase of the infection can be complete; although half of those who are afflicted die.

Yellow fever appears in human populations in different ways. One pattern of appearance is called sylvatic (or jungle) yellow fever. As the name implies, this form is restricted to regions that are largely uninhabited by humans. The virus cycles between the indigenous monkey population and the mosquitoes that bite them. Humans that enter the region, such as loggers, can become infected.

Another cycle of infection is referred to as intermediate yellow fever. This infection is found in semi-urban areas, such as where villages are separated by intervening areas of farmland or more natural areas. Infections can spring up in several areas simultaneously. Migration of people from the infected areas to larger population centers can spread the infection. This is the most common pattern of yellow fever occurring in present day Africa.

The final pattern of yellow fever is that which occurs in fully urban settings. The large population base can produce a

large epidemic. The infection is spread exclusively by mosquitoes feeding on one person then on another. Control of these **epidemics** concentrates on eradicating the mosquito populations.

Treatment for yellow fever consists primarily of keeping the patient hydrated and comfortable. Prevention of the infection, via **vaccination**, is the most prudent course of action. The current vaccine (which consists of living but weakened virus) is safe and provides long-lasting **immunity**. While side effects are possible, the risks of not vaccinating far outweigh the risk of the adverse vaccine reactions. For a vaccination campaign to be effective, over 80% of the people in a suspect region need to be vaccinated. Unfortunately few countries in Africa have achieved this level of coverage. Another course of action is the control of mosquito populations, typically by spraying with a compound that is toxic to mosquito larvae during breeding season. Once again, this coverage must be extensive to be successful. Breeding areas missed during spraying ensure the re-emergence of mosquitoes and, hence, of the yellow fever virus.

See also Transmission of pathogens; Zoonoses

Z

ZIEHL-NEELSEN STAIN • *see* LABORATORY TECHNIQUES IN MICROBIOLOGY

ZOBELL, CLAUDE EPHRAIM (1904-1989)

American microbiologist and marine biologist

Claude Ephraim ZoBell's research confirmed several behavioral characteristics of water and ocean-borne **bacteria**. ZoBell researched the special adhesive properties of organisms to surfaces, and experimented with mean of controlling such populations. He also was one of the pioneering scientists to study marine pollution. His work continues to be utilized by marine biologists, petroleum engineers, and the shipping industry.

ZoBell was born in Provo, Utah, but his family moved to Rigby, Idaho, when he was young. He pursued studies in biology and bacteriology at the University of California at Berkeley. By the time he was awarded his Ph.D. in 1931, he had already conducted several studies on the **biochemistry** of various bacteria and developed his interest in marine biology.

ZoBell's first position was as Instructor of **Marine Microbiology** at the Scripps Institute of Oceanography. He was made a full professor in 1948 after conducting research in environmental biology. While at the Scripps Institute, ZoBell left his research in medical microbiology in favor of pursuing his interests in marine life. Thus, ZoBell was among the first generations of modern marine biologists.

Most of ZoBell's career defining research was conducted while at Scripps. ZoBell noted that most of the research done at the institute focused on relationships between various groups of organisms, instead of trying to isolate various organisms in a specific environment. Also, he quickly found that he, as well as other marine scientists, were frustrated by difficulties in reproducing marine conditions and organism behavior and growth in the lab.

ZoBell and his colleagues devised a number of technical innovations and methodological procedures that help to overcome such obstacles to their research. For example, ZoBell designed a slide carrier that could be lowered into the water to study the attachment of organisms to surfaces, thus eliminating the need to **culture** or breed organisms in the lab. Organisms that colonized the slide carrier were removed from the water and instantly processed for microscopic observation. The device proved successful, eliminating the need for a multitude of culture media in the lab. This microscopic observation of cultured slides became known as biofilm microbiology.

ZoBell and his colleagues also conducted experiments on bacteria and organism levels in seawater. The scientists lowered a series of sterile glass bottles into the water, permitted water to flow in and out of the bottles for several days, and then raised the bottles. ZoBell found that bacterial levels were higher on the glass than in the liquid. Thus, ZoBell devised that certain organisms have a certain "sticking power" and prefer to colonize surfaces rather than remain free-floating. The experiment was repeated in the lab using seawater specimens, with similar results. The exact nature of this sticking power, be it with barnacles or bacteria, remains alusive.

After receiving several rewards for his research at the Scripps Institute for Oceanography, ZoBell briefly researched and taught at Princeton University, in Europe, and spent time at several other oceanographic research institutes. He returned to the Scripps Institute and turned his attention to the effects of pollution and petroleum drilling on marine environments. He remained a passionate advocate for marine preservation and research until his death.

See also Biofilm formation and dynamic behavior

ZOONOSES

Zoonoses are diseases of microbiological origin that can be transmitted from animals to people. The causes of the diseases can be **bacteria**, **viruses**, **parasites**, and **fungi**.

Sheep can act as host for a number of zoonotic disease pathogens.

Zoonoses are relevant for humans because of their species-jumping ability. Because many of the causative microbial agents are resident in domestic animals and birds, agricultural workers and those in food processing plants are at risk. From a research standpoint, zoonotic diseases are interesting as they result from organisms that can live in a host innocuously while producing disease upon entry into a different host environment.

Humans can develop zoonotic diseases in different ways, depending upon the microorganism. Entry through a cut in the skin can occur with some bacteria. Inhalation of bacteria, viruses, and fungi is also a common method of transmission. As well, the ingestion of improperly cooked food or inadequately treated water that has been contaminated with the fecal material from animals or birds present another route of disease transmission.

A classic historical example of a zoonotic disease is **yellow fever**. The construction of the Panama Canal took humans into the previously unexplored regions of the Central American jungle. Given the opportunity, transmission from the resident animal species to the newly arrived humans occurred. This phenomenon continues today. Two examples are illustrative of this. First, the clearing of the Amazonian rain forest to provide agricultural land has resulted in the emergence of Mayaro and Oropouche virus infections in the woodcutters. Second, in the mid 1990s, fatalities in the Southwestern United States were traced to the hanta virus that has been transmitted from rodents to humans.

A number of bacterial zoonotic diseases are known. A few examples are **Tularemia**, which is caused by *Francisella tulerensis*, Leptospirosis (*Leptospiras spp.*), **Lyme disease** (*Borrelia burgdorferi*), Chlaydiosis (*Chlamydia psittaci*), Salmonellosis (*Salmonella spp.*), **Brucellosis** (*Brucella melitensis, suis,* and *abortus*, Q-fever (*Coxiella burnetti*), and **Campylobacteriosis** (*Campylobacter jejuni*).

Zoonoses produced by fungi, and the organism responsible, include Aspergillosis (*Aspergillus fumigatus*). Well-known viral zoonoses include **rabies** and encephalitis. The **microorganisms** called Chlamydia cause a **pneumonia**-like disease called psittacosis.

Within the past two decades two protozoan zoonoses have definitely emerged. These are **Giardia** (also commonly known as "beaver fever"), which is caused by *Giardia lamblia* and **Cryptosporidium**, which is caused by *Cryptosporidium parvum*. These protozoans reside in many vertebrates, particularly those associated with wilderness areas. The increasing encroachment of human habitations with wilderness is bringing the animals, and their resident microbial flora, into closer contact with people.

Similarly, human encroachment is thought to be the cause for the emergence of devastatingly fatal viral **hemorrhagic fevers**, such as Ebola and Rift Valley fever. While the origin of these agents is not definitively known, zoonotic transmission is assumed.

In the present day, outbreaks of hoof and mouth disease among cattle and sheep in the United Kingdom (the latest being in 2001) has established an as yet unproven, but compelling, zoonotic link between these animals and humans, involving the disease causing entities known as **prions**. While the story is not fully resolved, the current evidence supports the transmission of the prion agent of mad cow disease to humans, where the similar brain degeneration disease is known as Creutzfeld-Jacob disease.

The increasing incidence of these and other zoonotic diseases has been linked to the increased ease of global travel. Microorganisms are more globally portable than ever before. This, combined with the innate ability of microbes to adapt to new environments, has created new combinations of microorganism and susceptible human populations.

See also Animal models of infection; Bacteria and bacterial infection

ZOOPLANKTON

Zooplankton are small animals that occur in the water column of either marine or freshwater ecosystems. Zooplankton are a diverse group defined on the basis of their size and function, rather than on their taxonomic affinities.

Most species in the zooplankton community fall into three major groups—Crustacea, Rotifers, and Protozoas. Crustaceans are generally the most abundant, especially those in the order Cladocera (waterfleas), and the class Copepoda (the copepods), particularly the orders Calanoida and Cyclopoida. Cladocerans are typically most abundant in fresh water, with common genera including Daphnia and Bosmina. Commonly observed genera of marine calanoid copepods include Calanus, Pseudocalanus, and Diaptomus, while abundant cyclopoid copepods include Cyclops and Mesocyclops. Other crustaceans in the zooplankton include species of opossum shrimps (order Mysidacea), amphipods (order Amphipoda), and fairy shrimp (order Anostraca). Rotifers (phylum Rotifera) are also found in the zooplankton, as are protozoans (kingdom Protista). Insects may also be important, especially in fresh waters close to the shoreline.

Most zooplankton are secondary consumers, that is, they are herbivores that graze on phytoplankton, or on unicel-

lular or colonial algae suspended in the water column. The productivity of the zooplankton community is ultimately limited by the productivity of the small algae upon which they feed. There are times when the biomass of the zooplankton at any given time may be similar to, or even exceed, that of the phytoplankton. This occurs because the animals of the zooplankton are relatively long-lived compared with the algal cells upon which they feed, so the turnover of their biomass is much less rapid. Some members of the zooplankton are detritivores, feeding on suspended organic detritus. Some species of zooplankton are predators, feeding on other species of zooplankton, and some spend part of their lives as **parasites** of larger animals, such as fish.

Zooplankton are important in the food webs of open-water ecosystems, in both marine and fresh waters. Zooplankton are eaten by relatively small fish (called planktivorous fish), which are then eaten by larger fish. Zooplankton are an important link in the transfer of energy from the algae (the primary producers) to the ecologically and economically important fish community (the consumers).

Species of zooplankton vary in their susceptibility to environmental stressors, such as exposure to toxic chemicals, acidification of the water, eutrophication and oxygen depletion, or changes in temperature. As a result, the species assemblages (or communities) of zooplankton are indicators of environmental quality and ecological change.

See also Bioremediation; Indicator species; Water pollution and purification

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Historical Chronology

Editor's note: This is a historical chronology principally devoted to marking milestones in human scientific achievement and is intended to provide a valuable reference that will enable readers to relate dates and events mentioned in the text to the larger scope of related scientific achievement. Although mention is made of epidemics and pandemics, it is beyond the scope of this chronology to provide a comprehensive listing of such events.

ca. 10000 B.C.

Neolithic Revolution: transition from a hunting-and-gathering mode of food production to farming and animal husbandry, that is, the domestication of plants and animals.

ca. 3500 B.C.

Sumerians describe methods of managing the date harvest.

ca. 700 B.C.

The use of anatomical models is established in India.

ca. 600 B.C.

Thales, the founder of the Ionian school of Greek philosophy, identifies water as the fundamental element of nature. Other Ionian philosophers construct different theories about the nature of the Universe and living beings.

ca. 500 B.C.

Alcmaeon, Pythagorean philosopher and naturalist, pursues anatomical research and concludes that humans are fundamentally different from animals. He also differentiates arteries from veins. His work establishes the foundations of comparative anatomy.

ca. 450 B.C.

Empedocles, Greek philosopher, asserts that the Universe and all living things are composed of four fundamental elements: earth, air, fire, and water.

ca. 430 B.C.

Plague of Athens caused by unknown infectious agent. One third of the population (increased by those fleeing the Spartan army) die.

ca. 400 B.C.

Hippocrates, Greek physician, establishes a school of medicine on the Aegean island of Cos. According to Hippocratic medical tradition, the four humors that make up the human body correspond to the four elements that make up the Universe. Hippocrates suggests using the developing chick egg as a model for embryology, and notes that offspring inherit traits from both parents.

ca. 400 B.C.

The Greek philosopher Democritus argues that atoms are the building blocks of the Universe and all living things. Democritus was an early advocate of the preformation theory of generation (embryology).

ca. 350 B.C.

The Greek philosopher Aristotle attempts to classify animals and describes various theories of generation, including sexual, asexual, and spontaneous generation. Aristotle argues that the male parent contributes "form" to the offspring and the female parent contributes "matter." He discusses preformation and epigenesis as possible theories of embryological development, but argues that development occurs by epigenesis.

ca. 50 B.C.

Lucretius proposes a materialistic, atomistic theory of nature in his poem *On the Nature of Things*. He favors the preformation theory of embryological development.

ca. A.D. 70
Roman author and naturalist Pliny the Elder (A.D. 23–79) writes his influential *Natural History*, a vast compilation combining observations of nature, scientific facts, and mythology. Naturalists will use his work as a reference book for centuries.

ca. 160 Bubonic plague (termed "barbarian boils") sweeps China.

ca. 166 Antonine plague in Rome (possibly smallpox or bubonic plague) eventually kills millions throughout the weakening Roman empire.

ca. 200 Galen, the preeminent medical authority of late Antiquity and the Middle Ages, creates a philosophy of medicine, anatomy, and physiology that remains virtually unchallenged until the sixteenth and seventeenth centuries. Galen argues that embryological development is epigenetic, although he disagrees with Aristotle about which organs are formed first and which are most important.

529 Byzantine Emperor Justinian closes the Academy in Athens that was founded by Plato and forbids pagan scientists and philosophers to teach. This causes an exodus of scientists to Persia.

ca. 980 Abu Al-Qasim Al-Zahravi (Abucasis) creates a system and method of human dissection along with the first formal specific surgical techniques.

ca. 1150
Hildegard of Bingen (1098–1179), Germanic author, publishes *The Book of Simple Medicine*, a treatise on the medicinal qualities of plants and minerals.

ca. 1267
Roger Bacon (1214–1292), English philosopher and scientist, asserts that natural phenomena should be studied empirically.

ca. 1275
William of Saliceto creates the first established record of a human dissection.

1348 The beginning of a three-year epidemic caused by *Yersinia pestis* that kills almost one-third of the population of urban Europe. In the aftermath of the epidemic, measures are introduced by the Italian government to improve public sanitation, marking the origin of public health.

1490 Leonardo da Vinci (1452–1519), Italian artist and scientist, describes patterns of capillary action.

1492 Venereal diseases, smallpox, and influenza brought by Columbus's expedition (and subsequent European explorers) to the New World. Millions of native peoples eventually die from these diseases because of a lack of prior exposure to stimulate immunity. In some regions, whole villages are wiped-out; and across broader regions, up to 95% of the native population dies as a result of exposure to these new pathogens.

ca. 1525
Paracelsus (1493–1541), Swiss physician and alchemist, uses mineral substances as medicines. Denying Galen's authority, Paracelsus teaches that life is a chemical process.

1542 Bubonic plague from China devastates Constantinople before advancing to repeatedly kill millions across Europe.

1543 Andreas Vesalius publishes his epoch-making treatise *The Fabric of the Human Body*. Vesalius generally accepts Galenic physiological doctrines and ideas about embryology, but corrects many of Galen's misconceptions regarding the human body. Vesalius is subsequently recognized as the founder of modern anatomy.

1546 Gerolamo Fracastoro (1478–1553) writes a treatise on contagious diseases that identifies and names syphilis. He presents a rudimentary concept of the germ theory of disease.

1568 Zacharias and Hans Janssen develop the first compound microscope. The innovation opens new opportunities for the study of structural detail.

1600 Girolamo Fabrizzi (Fabricus ab Aquapendente) publishes *De formato foetu* (On the formation of the fetus). The illustrations stir academic debate.

1604 German astronomer and mathematician Johannes Kepler (1571–1630) writes a treatise on optics.

1610 Jean Beguin (1550–1620) publishes the first textbook on chemistry.

1614 Italian physician Santorio Santorio (1561–1636) publishes studies on metabolism.

1628 William Harvey (1578–1657), English physician, publishes his *Anatomical Treatise on the Movement of the Heart and Blood*. This scientific classic presents the first accurate description of blood circulation, tracing the course of blood through the heart, arteries, and veins.

1651 Harvey publishes *On the Generation of Animals,*, a treatise on embryology in which Harvey asserts that all living things come from eggs. He argues that oviparous and viviparous generation are analogous, but maintains support for the Aristotelian doctrine that generation occurs by epigenesis.

1658 Dutch naturalist Jan Swammerdam publishes records of observations of red blood cells.

1660 Marcello Malpighi publishes his observations concerning vascular capillary beds and individual capillaries.

1664 René Descartes (1596–1650), French philosopher and mathematician, publicizes his idea of reflexive action. The assertion is included in a French edition of his posthumously published work on animal physiology. In his analysis Descartes applies his mechanistic philosophy to the analysis of animal behavior; he first uses the concept of reflex to denote any involuntary response the body makes when exposed to a stimulus.

1665 Robert Hooke publishes *Micrographia,* an account of observations made with the new instrument known as the microscope. Hooke presents his drawings of the tiny box-like structures found in cork and calls these tiny structures "cells." Although the cells he observes are not living, the name is retained. He also describes the streaming juices of live plant cells.

1668 Francesco Redi publishes *Experiments on the Generation of Insects*, in which he demonstrates that maggots develop from eggs laid by flies. His observations disprove the theory that maggots are spontaneously generated from rotting meats.

1669 Jan Swammerdam begins his pioneering work on the metamorphosis of insects and the anatomy of the mayfly. Swammerdam suggests that new individuals were embedded, or preformed, in their predecessors. Nicolas de Malebranche later reformulates Swammerdam's preformationist ideas into a more sophisticated philosophical doctrine that involves a series of embryos preexisting within each other like a nest of boxes.

1674 Antoni van Leeuwenhoek observes "animalcules" in lake water viewed through a ground glass lens. This observation of what will eventually be known as bacteria represents the start of the formal study of microbiology.

1683 Antoni van Leeuwenhoek discovers different types of minute organisms he refers to as "infusoria" in decomposing matter and stagnant water. He also describes protozoa and bacteria.

1700 Joseph Pitton de Tournefort presents an early version of the binomial method of classification, which is subsequently developed by Carl Linnaeus.

1727 Hales studies plant nutrition and measures water absorbed by plant roots and released by leaves. He argues that something in the air (carbon dioxide) is converted into food, and that light is a necessary element of this process.

1735 Carl Linnaeus publishes his *Systema Naturae, or The Three Kingdoms of Nature Systematically Proposed in Classes, Orders, Genera, and Species*,, a methodical and hierarchical classification of all living beings. He develops the binomial nomenclature for the classification of plants and animals. In this system, each type of living being is classified in terms of genus (denoting the group to which it belongs) and species (its particular, individual name). His classification of plants is primarily based on the characteristics of their reproductive organs.

1740 Abraham Trembley asserts that the fresh water hydra, or "polyp," appears to be an animal rather than a plant. When the hydra is cut into pieces, each part regenerates a complete new organism. These experiments raise many philosophical questions about the "organizing principle" in animals and the nature of development.

1746 Pierre-Louis Moreau de Maupertuis publishes *Venus Physique*. Maupertuis criticizes preformationist theories because offspring inherit characteristics of both parents. He proposes an adaptationist account of organic design. His theories suggests the existence of a mechanism for transmitting adaptations.

1748 Nollet describes osmosis.

1754 Pierre-Louis Moreau de Maupertuis suggests that species change over time, rather than remaining fixed.

1757 Albrecht von Haller (1757–1766) publishes the first volume of his eight-volume *Elements of Physiology of the Human Body*, subsequently to become a landmark in the history of modern physiology.

1759 Kaspar Friedrich Wolff publishes *Theory of Generation,* which argues that generation occurs by epigenesis (the gradual addition of parts). This book marks the beginning of modern embryology.

1762 Marcus Anton von Plenciz, Sr. suggests that all infectious diseases are caused by living organisms and that there is a particular organism for each disease.

1765 Abraham Trembley observes and publishes drawings of cell division in protozoans and algae.

1765 Lazzaro Spallanzani publishes his *Microscopical Observations*. Spallanzani's experiments refutes the theory of the spontaneous generation of infusoria.

1772 Joseph Priestley (1733–1804), an English theologian and chemist, discovers that plants give off oxygen.

1774 Antoine-Laurent Lavoisier (1743–1794), a French chemist, discovers that oxygen is consumed during respiration.

1779 Jan Ingenhousz (1739–1799), Dutch physician and plant physiologist, publishes his *Experiments upon Vegetables*. He shows that light is necessary for the

production of oxygen, and that carbon dioxide is taken in by plants in the daytime and given off at night.

1780 Antoine-Laurent Lavoisier (1743–1794), French chemist, and Pierre-Simon Laplace (1749–1827), French astronomer and mathematician, collaborate to demonstrate that respiration is a form of combustion. Breathing, like combustion, liberates heat, carbon dioxide, and water.

1780 George Adams (1750–1795), English engineer, engineers the first microtome. This mechanical instrument cuts thin slices for examination under a microscope, thus replacing the imprecise procedure of cutting with a hand-held razor.

1789 Antoine-Laurent de Jussieu publishes his *Plant Genera,*, a widely acclaimed book that incorporates the Linnaean system of binomial nomenclature. This book comes to be regarded as the foundation of the natural system of botanical classification. Jussieu classifies plants on the basis of cotyledons, and divides all plants into acotyledons, monocotyledons, and dicotyledons.

1796 Edward Jenner (1749–1823) uses cowpox virus to develop a smallpox vaccine.

1796 Erasmus Darwin, grandfather of Charles Darwin and Francis Galton, publishes his *Zoonomia*. In this work, Darwin argues that evolutionary changes are brought about by the mechanism primarily associated with Jean-Baptiste Lamarck, i.e., the direct influence of the environment on the organism.

1797 Georges-Léopold-Chrétien-Frédéric Dagobert Cuvier establishes modern comparative zoology with the publication of his first book, *Basic Outline for a Natural History of Animals*. Cuvier studies the ways in which an animal's function and habits determine its form. He argues that form always followed function and that the reverse relationship did not occur.

1798 Government legislation is passed to establish hospitals in the United States devoted to the care of ill mariners. This initiative leads to the establishment of a Hygenic Laboratory that eventually grows to become the National Institutes of Health.

1800 Marie-François-Xavier Bichat publishes his first major work, *Treatise on Tissues,* which establishes histology as a new scientific discipline. Bichat distinguishes 21 kinds of tissue and relates particular diseases to particular tissues.

1802 Jean-Baptiste-Pierre-Antoine de Monet de Lamarck and Gottfried Reinhold Treviranus propose the term "biology" to denote a new general science of living beings that would supercede studies in natural history.

1802 John Dalton introduces modern atomic theory into the science of chemistry.

1809 Jean-Baptiste-Pierre-Antoine de Monet de Lamarck introduces the term "invertebrate" in his *Zoological Philosophy,*, which contains the first influential scientific theory of evolution. He attempts to classify organisms by function rather than by structure and is the first to use genealogical trees to show relationships among species.

1812 Kirchoff identifies catalysis and mechanisms of catalytic reactions.

1817 Georges-Léopold-Chrétien-Frédéric Dagobert Cuvier publishes his major work, *The Animal Kingdom,* which expands and improves Linnaeus's classification system. Cuvier groups related classes into a broader category called a phylum. He is also the first to extend this system of classification to fossils.

1818 William Charles Wells suggests the theory of natural selection in an essay dealing with human color variations. He notes that dark skinned people are more resistant to tropical diseases than lighter skinned people. Wells also calls attention to selection carried out by animal breeders. Jerome Lawrence, James Cowles Prichard, and others make similar suggestions, but do not develop their ideas into a coherent and convincing theory of evolution.

1820 First United States *Pharmacopoeia* is published.

1824 René–Joachim-Henri Dutrochet suggests that tissues are composed of living cells.

1826 James Cowles Prichard presents his views on evolution in the second edition of his book *Researches into the Physical History of Man* (first edition 1813). These ideas about evolution are suppressed in later editions.

1828 Friedrich Wöhler synthesizes urea. This is generally regarded as the first organic chemical produced in the laboratory, and an important step in disproving the idea that only living organisms can produce organic compounds. Work by Wöhler and others establish the foundations of organic chemistry and biochemistry.

1828 Karl Ernst von Baer publishes a book entitled *On the Developmental History of Animals* (2 volumes, 1828–1837), in which he demonstrates that embryological development follows essentially the same pattern in a wide variety of mammals. Early mammalian embryos are very similar, but they diverge at later stages of gestation. Von Baer's work establishes the modern field of comparative embryology.

1828 Robert Brown observes a small body within the cells of plant tissue and calls it the "nucleus." He

also discovers what becomes known as "Brownian movement."

1831 Charles Robert Darwin begins his historic voyage on the H.M.S. *Beagle* (1831–1836). His observations during the voyage lead to his theory of evolution by means of natural selection.

1831 Patrick Matthew includes a discussion of evolution and natural selection in his book *On Naval Timber and Arboriculture*. Matthew later claims priority in the discovery of evolution by means of natural selection in an article published in 1860 in the journal *Gardeners' Chronicle*.

1832 The French physiologist Anselme Payen (1795–1871) isolates diastase from barley. Diastase catalyzes the conversion of starch into sugar, and is an example of the organic catalysts within living tissue that eventually come to be called enzymes.

1836 Félix Dujardin describes the "living jell" of the cytoplasm, which he calls "sarcode."

1836 Theodor Schwann carries out experiments that refute the theory of the spontaneous generation of infusoria. He also demonstrates that alcoholic fermentation depends on the action of living yeast cells. The same conclusion is reached independently by Charles Caignard de la Tour.

1837 French physiologist René–Joachim Dutrochet (1776–1847) publishes his research on plant physiology that includes pioneering work on osmosis. He is the first scientist to systematically investigate the process of osmosis, which he names, and to argue that chlorophyll is necessary for photosynthesis.

1838 Matthias Jakob Schleiden notes that the nucleus first described by Robert Brown is a characteristic of all plant cells. Schleiden describes plants as a community of cells and cell products. He helps establish cell theory and stimulates Theodor Schwann's recognition that animals are also composed of cells and cell products.

1839 Jan Evangelista Purkinje uses the term "protoplasm" to describe the substance within living cells.

1839 Theodore Schwann extends the theory of cells to include animals and helps establish the basic unity of the two great kingdoms of life. He publishes *Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants*, in which he asserts that all living things are made up of cells and that each cell contains certain essential components. He also coins the term "metabolism" to describe the overall chemical changes that take place in living tissues.

1840 Friedrich Gustav Jacob Henle publishes the first histology textbook, *General Anatomy*. This work includes the first modern discussion of the germ theory of communicable diseases.

1840 German chemist Justus von Liebig (1803–1873) shows that plants synthesize organic compounds from carbon dioxide in the air but take their nitrogenous compounds from the soil. He also states that ammonia (nitrogen) is needed for plant growth.

1840 Karl Bogislaus Reichert introduces the cell theory into the discipline of embryology. He proves that the segments observed in fertilized eggs develop into individual cells, and that organs develop from cells.

1842 Charles Robert Darwin writes out an abstract of his theory of evolution, but he does not plan to have this theory published until after his death.

1842 Theodor Ludwig Wilhelm Bischoff publishes the first textbook of comparative embryology, *Developmental History of Mammals and Man.*

1844 Robert Chambers anonymously publishes *Vestiges of the Natural History of Creation,* which advocates the theory of evolution. This controversial book becomes a best seller and introduces the general reading public to the theory of evolution.

1845 Karl Theodor Ernst von Siebold realizes that protozoa are single-celled organisms. He is the first scientist to define protozoa as organisms.

1847 A series of yellow fever epidemics sweeps the American Southern states. The epidemics recur every few years for more than 30 years.

1851 Hugo von Mohl publishes his *Basic Outline of the Anatomy and Physiology of the Plant Cell*, in which he proposes that new cells are created by cell division.

1854 Gregor Mendel begins to study 34 different strains of peas. He selects 22 kinds for further experiments. From 1856 to 1863, Mendel grows and tests over 28,000 plants and analyzes seven pairs of traits.

1855 Alfred Russell Wallace writes an essay entitled *On the Law Which has Regulated the Introduction of New Species* and sends it to Charles Darwin. Wallace's essay and one by Darwin are published in the 1858 *Proceedings of the Linnaean Society*.

1857 Louis Pasteur demonstrates that lactic acid fermentation is caused by a living organism. Between 1857 and 1880 he performs a series of experiments that refute the doctrine of spontaneous generation. He also introduces vaccines for fowl cholera, anthrax, and rabies, based on attenuated strains of viruses and bacteria.

1858 Charles Darwin and Alfred Russell Wallace agree to a joint presentation of their theory of evolution by natural selection.

1858 Rudolf Ludwig Carl Virchow publishes his landmark paper "Cellular Pathology," thus establishing the field of that name. Virchow asserts that all cells arise from preexisting cells (*Omnis cellula e cellula*). He argues that the cell is the ultimate locus of all disease.

1859 Charles Robert Darwin publishes his landmark book *On the Origin of Species by Means of Natural Selection.*

1860 Ernst Heinrich Haeckel describes the essential elements of modern zoological classification.

1860 Louis Pasteur carries out experiments that disprove the doctrine of spontaneous generation.

1860 Max Johann Sigismund Schultze describes the nature of protoplasm and shows that it is fundamentally the same for all life forms.

1863 Thomas Henry Huxley publishes *Evidence As to Man's Place in Nature*, which extends Darwin's theory of evolution to include humans. Huxley becomes the champion and defender of Darwinism in England.

1865 An epidemic of rinderpest kills 500,000 cattle in Great Britain. Government inquiries into the outbreak pave the way for the development of contemporary theories of epidemiology and the germ theory of disease.

1865 Gregor Mendel presents his work on hybridization of peas to the Natural History Society of Brno, Czechoslovakia. The paper is published in the 1866 issue of the Society's *Proceedings*. Mendel presents statistical evidence that hereditary factors are inherited from both parents in a series of papers on "Experiments on Plant Hybridization" published between 1866 and 1869. Although his experiments provide evidence of dominance, the laws of segregation, and independent assortment, his work is generally ignored until 1900.

1866 Ernst Heinrich Haeckel publishes his book *A General Morphology of Organisms*. Haeckel summarizes his ideas about evolution and embryology in his famous—though long-discredited—dictum "ontogeny recapitulates phylogeny" (or, the development of an individual organism follows the same stages as the development of its species). He suggests that the nucleus of a cell transmits hereditary information and introduces the term "ecology" to describe the study of living organisms and their interactions with other organisms and with their environment.

1866 The Austrian botanist and monk Johann Gregor Mendel (1822–1884) discovers the laws of heredity and writes the first of a series of papers on the subject (1866–1869). The papers formulate the laws of hybridization. Mendel's work is disregarded until 1900, when de Vries rediscovers it. Unbeknownst to both Darwin and Mendel, Mendelian laws provide the scientific framework for the concepts of gradual evolution and continuous variation.

1867 Robert Koch establishes the role of bacteria in anthrax, providing the final piece of evidence in support of the germ theory of disease. Koch goes on to formulate postulates that, when fulfilled, confirm bacteria or viruses as the cause of an infection.

1868 Charles Darwin publishes *The Variation of Animals and Plants under Domestication* (2 volumes).

1868 Thomas Henry Huxley introduces the term "protoplasm" to the general public in a lecture entitled "The Physical Basis of Life."

1869 Johann Friedrich Miescher discovers nuclein, a new chemical isolated from the nuclei of pus cells. Two years later he isolates nuclein from salmon sperm. This material comes to be known as nucleic acid.

1870 Thomas Huxley delivers a speech that introduces the terms biogenesis (life from life) and abiogenesis (life from non-life; spontaneous generation). The speech strongly supports Pasteur's claim to have refuted the concept of spontaneous generation.

1871 Charles Robert Darwin publishes *The Descent of Man, and Selection in Relation to Sex*. This work introduces the concept of sexual selection and expands his theory of evolution to include humans.

1871 Ferdinand Julius Cohn coins the term bacterium.

1872 Franz Anton Schneider observes and describes the behavior of nuclear filaments (chromosomes) during cell division in his study of the platyhelminth Mesostoma. His account is the first accurate description of the process of mitosis in animal cells.

1873 Camilo Golgi discovers that tissue samples can be stained with an inorganic dye (silver salts). Golgi uses this method to analyze the nervous system and characterizes the cells known as Golgi Type I and Golgi Type II cells and the "Golgi Apparatus." Golgi is subsequently awarded a Nobel Prize in 1906 for his studies of the nervous system.

1873 Franz Anton Schneider describes cell division in detail. His drawings include both the nucleus and chromosomal strands.

1873 Walther Flemming discovers chromosomes, observes mitosis, and suggests the modern interpretation of nuclear division.

1874 Wilhelm August Oscar Hertwig concludes that fertilization in both animals and plants consists of the physical union of the two nuclei contributed by the male and female parents. Hertwig subsequently carries out pioneering studies of reproduction of the sea urchin.

1875 Eduard Adolf Strasburger publishes *Cell-Formation and Cell-Division*, in which he describes nuclear division in plants. Strasburger accurately describes the process of mitosis and argues that new nuclei can only rise from the division of preexisting nuclei. His treatise helps establish cytology as a distinct branch of histology.

1875 Ferdinand Cohn publishes a classification of bacteria in which the genus name *Bacillus* is used for the first time.

1876 Edouard G. Balbiani observes the formation of chromosomes.

1876 Robert Koch publishes a paper on anthrax that implicates a bacterium as the cause of the disease, validating the germ theory of disease

1877 Robert Koch describes new techniques for fixing, staining, and photographing bacteria.

1877 Paul Erlich recognizes the existence of the mast cells of the immune system.

1877 Wilhelm Friedrich Kühne proposes the term enzyme (meaning "in yeast"). Kühne establishes the critical distinction between enzymes, or "ferments," and the microorganisms that produce them.

1878 Charles-Emanuel Sedillot introduces the term "microbe." The term becomes widely used as a term for a pathogenic bacterium.

1878 Joseph Lister publishes a paper describing the role of a bacterium he names *Bacterium lactis* in the souring of milk.

1878 Thomas Burrill demonstrates for the first time that a plant disease (pear blight) is caused by a bacterium (*Micrococcus amylophorous*).

1879 Albert Nisser identifies *Neiserria gonorrhoeoe* as the cause of gonorrhea.

1879 Walther Flemming describes and names chromatin, mitosis, and the chromosome threads. Fleming's drawings of the longitudinal splitting of chromosomes in eukaryotic cells provide the first accurate counts of chromosome numbers.

1880 C. L. Alphonse Laveran isolates malarial parasites in erythrocytes of infected people and demonstrates that the organism can replicate in the cells. He is awarded the 1907 Nobel Prize in Medicine or Physiology for this work.

1880 Louis Pasteur develops a method of weakening a microbial pathogen of chicken, and uses the term "attenuated" to describe the weakened microbe.

1880 The first issue of the journal Science is published by the American Association for the Advancement of Science.

1880 Walther Flemming, Eduard Strasburger, Edouard van Beneden, and others document the basic outlines of cell division and the distribution of chromosomes to the daughter cells.

1881 Eduard Strasburger coins the terms cytoplasm and nucleoplasm.

1881 Walther Flemming discovers the lampbrush chromosomes.

1882 Angelina Fannie and Walter Hesse in Koch's laboratory develop agar as a solid growth medium for microorganisms. Agar replaces gelatin as the solid growth medium of choice in microbiology.

1882 Edouard van Beneden outlines the principles of genetic continuity of chromosomes in eukaryotic cells and reports the occurrence of chromosome reduction during the formation of the germ cells.

1882 Pierre Émile Duclaux suggest that enzymes should be named by adding the suffix "ase" to the name of their substrate.

1882 The German bacteriologist Robert Koch (1843–1910) discovers the tubercle bacillus and enumerates "Koch's postulates," which define the classic method of preserving, documenting, and studying bacteria.

1882 Walther Flemming publishes *Cell Substance, Nucleus, and Cell Division*, in which he describes his observations of the longitudinal division of chromosomes in animal cells. Flemming observes chromosome threads in the dividing cells of salamander larvae.

1882 Wilhelm Roux offers a possible explanation for the function of mitosis.

1883 August F. Weismann begins work on his germplasm theory of inheritance. Between 1884 and 1888, Weismann formulates the germplasm theory that asserts that the germplasm was separate and distinct from the somatoplasm. He argues that the germplasm was continuous from generation to generation and that only changes in the germplasm were transmitted to further generations. Weismann proposes a theory of chromosome behavior during cell division and fertilization and predicts the occurrence of a reduction division (meiosis) in all sexual organisms.

1883 Edward Theodore Klebs and Frederich Loeffler independently discover *Corynebacterium diphtheriae*, the bacterium that causes diphtheria.

1883 Walther Flemming, Eduard Strasburger and Edouard Van Beneden demonstrate that, in eukaryotic cells, chromosome doubling occurs by a process of longitudinal splitting. Strasburger describes and names the prophase, metaphase, and anaphase stages of mitosis.

1883 Wilhelm Roux suggests that chromosomes carry the hereditary factors.

1884 Elie Metchnikoff discovers the antibacterial activity of white blood cells, which he calls "phagocytes," and formulates the theory of phagocytosis. He also develops the cellular theory of vaccination.

1884 Hans Christian J. Gram develops the Gram stain.

1884 Louis Pasteur and coworkers publish a paper entitled "A New Communication on Rabies." Pasteur proves that the causal agent of rabies can be attenuated and the weakened virus can be used as a vaccine to prevent the disease. This work serves as the basis of future work on virus attenuation, vaccine development, and the concept that variation is an inherent characteristic of viruses.

1884 Oscar Hertwig, Eduard Strasburger, Albrecht von Kölliker, and August Weismann independently report that the cell nucleus serves as the basis for inheritance.

1885 Francis Galton devise a new statistical tool, the correlation table.

1885 French chemist Louis Pasteur (1822–1895) inoculates a boy, Joseph Meister, against rabies. Meister had been bitten by an infected dog. The treatment saves his life. This is the first time Pasteur uses an attenuated germ on a human being.

1885 Paul Ehrlich proposes that certain chemicals such as arsenic are toxic to bacteria.

1885 Theodor Escherich identifies a bacterium inhabiting the human intestinal tract that he names *Bacterium coli* and shows that the bacterium causes infant diarrhea and gastroenteritis. The bacterium is subsequently named *Escherichia coli*.

1886 Adolf Mayer publishes the landmark article "Concerning the Mosaic Disease of Tobacco." This paper is considered the beginning of modern experimental work on plant viruses. Mayer assumes that the causal agent is a bacterium, though he is unable to isolate it.

1887 Julius Richard Petri develops a culture dish that has a lid to exclude airborne contaminants. The innovation is subsequently termed the Petri plate.

1888 Heinrich Wilhelm Gottfried Waldeyer coins the term "chromosome." Waldeyer also introduces the use of hematoxylin as a histological stain.

1888 Martinus Beijerinck uses a growth medium enriched with certain nutrients to isolate the bacterium *Rhizobium*, demonstrating that nutritionally tailored growth media are useful in bacterial isolation.

1888 The Institute Pasteur is formed in France.

1889 Richard Altmann develops a method of preparing nuclein that is apparently free of protein. He calls his protein-free nucleins "nucleic acids."

1889 Theodor Boveri and Jean-Louis-Léon Guignard establish the numerical equality of the paternal and maternal chromosomes at fertilization.

1891 Charles-Edouard Brown-Sequard suggests the concept of internal secretions (hormones).

1891 Paul Ehrlich proposes that antibodies are responsible for immunity.

1891 Robert Koch proposes the concept of delayed type hypersensitivity.

1892 August Weismann publishes his landmark treatise *The Germ Plasm: A Theory of Heredity*, which emphasizes the role of meiosis in the distribution of chromosomes during the formation of gametes.

1892 Dmitri Ivanowski demonstrates that filterable material causes tobacco mosaic disease. The infectious agent is subsequently showed to be the tobacco mosaic virus. Ivanowski's discovery creates the field of virology.

1892 George M. Sternberg publishes his *Practical Results of Bacteriological Researches*. Sternberg's realization that a specific antibody was produced after infection with vaccinia virus and that immune serum could neutralize the virus becomes the basis of virus serology. The neutralization test provides a technique for diagnosing viral infections, measuring the immune response, distinguishing antigenic similarities and differences among viruses, and conducting retrospective epidemiological surveys.

1893 William Bateson publishes *Materials for the Study of Variation*, which emphasizes the importance of discontinuous variations (the kinds of variation studied by Mendel).

1894 Alexandre Yersin isolates *Yersinia (Pasteurella) pestis*, the bacterium responsible for bubonic plague.

1894 Wilhelm Konrad Roentgen discovers x rays.

1897 John Jacob Abel (1857–1938), American physiologist and chemist, isolates epinephrine (adrenalin). This is the first hormone to be isolated.

1898 Carl Benda discovers and names mitochondria, the subcellular entities previously seen by Richard Altmann.

1898 Friedrich Loeffler and Paul Frosch publish their *Report on Foot-and-Mouth Disease*. They prove that this animal disease is caused by a filterable virus and suggests that similar agents might cause other diseases.

1898 Martin Wilhelm Beijerinck publishes his landmark paper "Concerning a Contagium Vivum Fluidum as Cause of the Spot Disease of Tobacco Leaves." Beijerinck thinks that the etiological agent, which could pass through a porcelain filter that removed known bacteria, might be a new type of invisible organism that reproduced within the cells of diseased plants. He realizes that a very small amount of the virus could infect many leaves and that the diseased leaves could infect others.

1898 The First International Congress of Genetics is held in London.

1899 A meeting to organize the Society of American Bacteriologists is held at Yale University. The society will later become the American Society for Microbiology.

1899 Jacques Loeb proves that it is possible to induce parthenogenesis in unfertilized sea urchin eggs by means of specific environmental changes.

1900 Carl Correns, Hugo de Vries, and Erich von Tschermak independently rediscover Mendel's laws of inheritance. Their publications mark the beginning of modern genetics. Using several plant species, de Vries and Correns perform breeding experiments that parallel Mendel's earlier studies and independently arrive at similar interpretations of their results. Therefore, upon reading Mendel's publication, they immediately recognized its significance. William Bateson describes the importance of Mendel's contribution in an address to the Royal Society of London.

1900 Hugo Marie de Vries describes the concept of genetic mutations in his book *Mutation Theory*. He uses the term mutation to describe sudden, spontaneous, drastic alterations in the hereditary material.

1900 Karl Landsteiner discovers the blood-agglutination phenomenon and the four major blood types in humans.

1900 Karl Pearson develops the chi-square test.

1900 Walter Reed demonstrates that Yellow Fever is caused by a virus transmitted by mosquitoes. This is the first demonstration of a viral cause of a human disease.

1900 Paul Erlich proposes the theory concerning the formation of antibodies by the immune system.

1901 Jules Bordet and Octave Gengou develop the complement fixation test.

1901 Theodor Boveri discovers that in order for sea urchin embryos to develop normally, they must have a full set of chromosomes. He concludes that the individual chromosomes must carry different hereditary determinants.

1901 William Bateson coins the terms genetics, F1and F2 generations, allelomorph (later shortened to allele), homozygote, heterozygote, and epistasis.

1902 Carl Neuberg introduces the term biochemistry.

1903 Archibald Edward Garrod provides evidence that errors in genes caused several hereditary disorders in human beings. His 1909 book *The Inborn Errors of Metabolism* is the first treatise in biochemical genetics.

1903 Ruska develops a primitive electron microscope.

1903 Tiselius offers electrophoresis techniques that become the basis for the separation of biological molecules by charge, mass, and size.

1903 Walter S. Sutton publishes a paper in which he presents the chromosome theory of inheritance. The theory, which states that the hereditary factors are located in the chromosomes, is independently proposed by Theodor Boveri and is generally referred to as the Sutton-Boveri hypothesis.

1906 Viennese physician and immunological researcher Clemens von Pirquet (1874–1929) coins the term allergy to describe the immune reaction to certain compounds.

1909 Phoebus Aaron Theodore Levene (1869–1940), Russian-American chemist, discovers the chemical difference between DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).

1909 Sigurd Orla-Jensen proposes that the physiological reactions of bacteria are primarily important in their classification.

1909 Wilhelm Ludwig Johannsen argues the necessity of distinguishing between the appearance of an organism and its genetic constitution. He invents the terms "gene" (carrier of heredity), "genotype" (an organism's genetic constitution), and "phenotype" (the appearance of the actual organism).

1911 Peyton Rous publishes the landmark paper "Transmission of a Malignant New Growth by Means of a Cell-Free Filtrate." His work provides the first rigorous proof of the experimental transmis-

sion of a solid tumor and suggests that a filterable virus is the causal agent.

1912 Casimir Funk (1884–1967), Polish-American biochemist, coins the term "vitamine." Since the dietary substances he discovers are in the amine group he calls all of them "life-amines" (using the Latin word *vita* for "life").

1912 The United States Public Health Service is established.

1912 Paul Ehrlich discovers a chemical cure for syphilis. This is the first chemotherapeutic agent for a bacterial disease.

1914 Frederick William Twort (1877–1950), English bacteriologist, and Felix H. D'Herelle (1873–1949), Canadian-Russian physician, independently discover bacteriophage.

1914 Thomas Hunt Morgan, Alfred Henry Sturtevant, Calvin Blackman Bridges, and Hermann Joseph Muller publish the classic treatise of modern genetics, *The Mechanism of Mendelian Heredity*.

1915 Frederick William Twort publishes the landmark paper "An Investigation of the Nature of Ultra-Microscopic Viruses." Twort notes the degeneration of bacterial colonies and suggests that the causative agent is an ultra-microscopic-filterable virus that multiplies true to type.

1915 Katherine K. Sanford isolates a single mammalian cell *in vitro* and allows it to propagate to form identical descendants. Her clone of mouse fibroblasts is called L929, because it took 929 attempts before a successful propagation was achieved. Sanford's work is an important step in establishing pure cell lines for biomedical research.

1916 Felix Hubert D'Herelle carries out further studies of the agent that destroys bacterial colonies and gives it the name "bacteriophage" (bacteria eating agent). D'Herelle and others unsuccessfully attempted to use bacteriophages as bactericidal therapeutic agents.

1917 D'Arcy Wentworth Thompson publishes *On Growth and Form,* which suggests that the evolution of one species into another occurs as a series of transformations involving the entire organism, rather than a succession of minor changes in parts of the body.

1918 Calvin B. Bridges discovers chromosomal duplications in *Drosophila*.

1918 More people are killed in a global influenza pandemic than soldiers die fighting World War I. By the end of 1918, approximately 25 million people die from a virulent strain of Spanish influenza.

1919 James Brown uses blood agar to study the destruction of blood cells by the bacterium *Streptococcus*. He observes three reactions that he designates alpha, beta, and gamma.

1919 The Health Organization of the League of Nations is established for the prevention and control of disease around the world.

1924 Albert Jan Kluyver publishes *Unity and Diversity in the Metabolism of Micro-organisms* He demonstrates that different microorganisms have common metabolic pathways of oxidation, fermentation, and synthesis of certain compounds. Kluyver also states that life on Earth depends on microbial activity.

1926 Bernard O. Dodge begins genetic studies on *Neurospora*.

1926 Thomas C. Vanterpool publishes a paper that clarifies the problem of "mixed infections" of plant viruses. His study of the condition known as "streak" or "winter blight" of tomatoes shows that it was the result of simultaneous infection of tomato plants by tomato mosaic virus and a potato mosaic virus.

1927 Hermann Joseph Muller induces artificial mutations in fruit flies by exposing them to x rays. His work proves that mutations result from some type of physical-chemical change. Muller goes on to write extensively about the danger of excessive x rays and the burden of deleterious mutations in human populations.

1927 Thomas Rivers publishes a paper that differentiates bacteria from viruses, establishing virology as a field of study that is distinct from bacteriology.

1928 Fred Griffith discovers that certain strains of pneumococci could undergo some kind of transmutation of type. After injecting mice with living R type pneumococci and heat-killed S type, Griffith is able to isolate living virulent bacteria from the infected mice. Griffith suggests that some unknown "principle" had transformed the harmless R strain of the pneumococcus to the virulent S strain.

1929 Francis O. Holmes introduces the technique of "local lesion" as a means of measuring the concentration of tobacco mosaic virus. The method becomes extremely important in virus purification.

1929 Frank M. Burnet and Margot McKie report critical insights into the phenomenon known as lysogeny (the inherited ability of bacteria to produce bacteriophage in the absence of infection). Burnet and McKie postulate that the presence of a "lytic unit" as a normal hereditary component of lysogenic bacteria. The "lytic unit" is proposed to be capable of liberating bacteriophage when it is activated by certain conditions. This concept is confirmed in the 1950s.

1929 Scottish biochemist Alexander Fleming (1881–1955) discovers penicillin. He observes that the mold *Penicillium notatum* inhibits the growth of some bacteria. This is the first anti-bacterial, and it opens a new era of "wonder drugs" to combat infection and disease.

1930 Curt Stern, and, independently, Harriet B. Creighton and Barbara McClintock, demonstrate cytological evidence of genetic crossing over between eukaryotic chromosomal strands.

1930 Max Theiler demonstrates the advantages of using mice as experimental animals for research on animal viruses. Theiler uses mice in his studies of the yellow fever virus.

1931 Phoebus A. Levene publishes a book that summarizes his work on the chemical nature of the nucleic acids. His analyses of nucleic acids seemed to support the hypothesis known as the tetranucleotide interpretation, which suggests that the four bases are present in equal amounts in DNAs from all sources. Perplexingly, this indicated that DNA is a highly repetitious polymer that is incapable of generating the diversity that would be an essential characteristic of the genetic material.

1932 William J. Elford and Christopher H. Andrewes develop methods of estimating the sizes of viruses by using a series of membranes as filters. Later studies prove that the viral sizes obtained by this method were comparable to those obtained by electron microscopy.

1934 John Marrack begins a series of studies that leads to the formation of the hypothesis governing the association between an antigen and the corresponding antibody.

1935 Wendall Meredith Stanley (1904–1971), American biochemist, discovers that viruses are partly protein-based. By purifying and crystallizing viruses, he enables scientists to identify the precise molecular structure and propagation modes of several viruses. Stanley wins the Nobel Prize in Chemistry in 1946.

1936 George P. Berry and Helen M. Dedrick report that the Shope virus could be "transformed" into Myxomatosis/Sanarelli virus. This virological curiosity was variously referred to as "transformation," "recombination," and "multiplicity of reactivation." Subsequent research suggests that it is the first example of genetic interaction between animal viruses, but some scientists warn that the phenomenon might indicate the danger of reactivation of virus particles in vaccines and in cancer research.

1936 Theodosius Dobzhansky publishes *Genetics and the Origin of Species,* a text eventually considered a classic in evolutionary genetics.

1937 Hans Adolf Krebs (1900–1981), German biochemist, describes and names the citric acid cycle.

1938 Emory L. Ellis and Max Delbrück perform studies on phage replication that mark the beginning of modern phage work. They introduce the "one-step growth" experiment, which demonstrates that after bacteriophages attack bacteria, replication of the virus occurs within the bacterial host during a "latent period," after which viral progeny are released in a "burst."

1939 Richard E. Shope reports that the swine influenza virus survived between epidemics in an intermediate host. This discovery is an important step in revealing the role of intermediate hosts in perpetuating specific diseases.

1939 Ernest Chain and H. W. Florey refine the purification of penicillin, making possible the mass production of the antibiotic.

1940 Ernest Chain and E. P. Abraham detail the inactivation of penicillin by a substance produced by *Escherichia coli*. This is the first bacterial compound known to produce resistance to an antibacterial agent.

1940 Helmuth Ruska obtains the first electron microscopic image of a virus.

1941 George W. Beadle and Edward L. Tatum publish their classic study on biochemical genetics entitled *Genetic Control of Biochemical Reactions in Neurospora*. Beadle and Tatum irradiate red bread mold *Neurospora* and prove that genes produce their effects by regulating particular enzymes. This work leads to the one gene–one enzyme theory.

1941 Lipmann describes and identifies the biochemical and physiological role of high energy phosphates (e.g., adenosine triphosphate; ATP).

1942 Jules Freund and Katherine McDermott identify adjuvants (e.g., paraffin oil) that act to boost antibody production.

1942 Salvador E. Luria and Max Delbrück demonstrate statistically that inheritance of genetic characteristics in bacteria follows the principles of genetic inheritance proposed by Charles Darwin. For their work the two (along with Alfred Day Hershey) are awarded the 1969 Nobel Prize in Medicine or Physiology.

1942 Salvador E. Luria and Thomas F. Anderson publish the first electron micrographs of bacterial viruses. The *Escherichia coli* bacteriophage appears to have a round, or polyhedral head and a thin tail.

1942 Selman Waksman suggests that the word "antibiotics" be used to identify antimicrobial compounds that are made by bacteria.

1944 New techniques and instruments, such as partition chromatography on paper strips and the photoelectric ultraviolet spectrophotometer, stimulate the development of biochemistry after World War II. New methodologies make it possible to isolate, purify, and identify many important biochemical substances, including the purines, pyrimidines, nucleosides, and nucleotides derived from nucleic acids.

1944 Oswald T. Avery, Colin M. MacLeod, and Maclyn McCarty publish a landmark paper on the pneumococcus transforming principle. The paper is entitled "Studies on the chemical nature of the substance inducing transformation of pneumococcal types." Avery suggests that the transforming principle seems to be deoxyribonucleic acid (DNA), but contemporary ideas about the structure of nucleic acids suggest that DNA does not possess the biological specificity of the hypothetical genetic material.

1944 Salvador E. Luria and Alfred Day Hershey prove that mutations occur in bacterial viruses, and they develop methods to distinguish the mutations from other alterations.

1945 Joshua Lederberg and Edward L. Tatum demonstrate genetic recombination in bacteria.

1945 Max Delbrück organizes the first session of the phage course at Cold Spring Harbor Laboratory. The widely influential phage course, which is subsequently taught for 26 consecutive years, serves as the training center for the first two generations of molecular biologists

1946 James B. Sumner, John H. Northrop, and Wendell M. Stanley receive the Nobel Prize in Chemistry for their independent work on the purification and crystallization of enzymes and viral proteins.

1946 Joshua Lederberg and Edward L. Tatum demonstrate that genetic recombination occurs in bacteria as the result of sexual mating. Lederberg and Tatum announce their discovery at the 1946 Cold Spring Harbor Symposium on Microbial Genetics, an event that becomes recognized as a landmark in the development of molecular biology.

1946 Max Delbrück and W. T. Bailey, Jr. publish a paper entitled "Induced Mutations in Bacterial Viruses." Despite some confusion about the nature of the phenomenon in question, this paper establishes the fact that genetic recombinations occur during mixed infections with bacterial viruses. Alfred Hershey and R. Rotman make the discovery of genetic recombination in bacteriophage simultaneously and independently. Hershey and his colleagues prove that this phenomenon can be used for genetic analyses. They construct a genetic map of phage particles and show that phage genes can be arranged in a linear fashion.

1947 Joshua Lederberg and Norton Zinder, and, independently, Bernard D. Davis, develop the penicillin-selection technique for isolating biochemically deficient bacterial mutants.

1948 Barbara McClintock publishes her research on transposable regulatory elements ("jumping genes") in maize. Her work was not appreciated until similar phenomena were discovered in bacteria and fruit flies in the 1960s and 1970s. McClintock was awarded the Nobel Prize in Medicine or Physiology in 1983.

1948 World Health Organization is formed. The WHO subsequently becomes the principle international organization managing public health related issues on a global scale. Headquartered in Geneva, the WHO becomes, by 2002, an organization of more than 190 member countries. The organization contributes to international public health in areas including disease prevention and control, promotion of good health, addressing disease outbreaks, initiatives to eliminate diseases (e.g., vaccination programs), and development of treatment and prevention standards.

1949 John F. Ender, Thomas H. Weller, and Frederick C. Robbins publish "Cultivation of Polio Viruses in Cultures of Human Embryonic Tissues." The report is a landmark in establishing techniques for the cultivation of poliovirus in cultures on non-neural tissue and for further virus research. The technique leads to the polio vaccine and other advances in virology.

1949 The role of mitochondria is finally revealed. These slender filaments within the cell, which participate in protein synthesis and lipid metabolism, are the cell's source of energy.

1949 Macfarlane Burnet and his colleagues begin studies that lead to the immunological tolerance hypothesis and the clonal selection theory. Burnet receives the 1960 Nobel Prize in Physiology or Medicine for this research.

1950 British physician Douglas Bevis demonstrates that amniocentesis could be used to test fetuses for Rh-factor incompatibility.

1950 Erwin Chargaff demonstrates that the Tetranucleotide Theory is incorrect and that DNA is more complex than the model developed by Phoebus A. Levene. Chargaff proves that the nucleic acids are not monotonous polymers. Chargaff also discovers interesting regularities in the base composition of DNA; these findings are later known as "Chargaff's rules." Chargaff discovers a one-to-one ratio of adenine to thymine and guanine to cytosine in DNA samples from a variety of organisms.

1950 Robert Hungate develops the roll-tube culture technique, which is the first technique that allows anaerobic bacteria to be grown in culture.

1950 Ruth Sager's work on the algae *Chlamydomonas* proves that cytoplasmic genes exist and that they can undergo mutation. She shows that such genes can be mapped on a "cytoplasmic chromosome." Confirmation is provided when other researchers report similar findings in yeast and *Neurospora,*. Subsequently the DNA is shown to be associated with cytoplasmic organelles.

1951 Esther M. Lederberg discovers a lysogenic strain of *Escherichia coli* K12 and isolates a new bacteriophage, called lambda.

1951 Rosalind Franklin obtains sharp x-ray diffraction photographs of deoxyribonucleic acid.

1952 Alfred Hershey and Martha Chase publish their landmark paper "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage." The famous "blender experiment" suggests that DNA is the genetic material. When bacteria are infected by a virus, at least 80% of the viral DNA enters the cell and at least 80% of the viral protein remains outside.

1952 James T. Park and Jack L. Strominger demonstrate that penicillin blocks the synthesis of the peptidoglycan of bacteria. This represents the first demonstration of the action of a natural antibiotic.

1952 Joshua Lederberg and Norton Zinder report the discovery of a phenomenon they call "transduction." Lederberg and Zinder prove that transduction in *Salmonella* is caused by phage particles that occasionally carry assorted host genes into new hosts (i.e., bacteriophage particles serve as the vectors of genetic exchange). The discovery of transduction is announced at Cold Spring Harbor in 1951. The next year, Zinder and Lederberg publish their results in a paper entitled "Genetic Exchange in Salmonella. New mechanism for the heritable transfer of genetic traits from one bacterial strain to another."

1952 Joshua Lederberg coins the term "plasmid" to describe genetic material that is capable of replicating but is not part of the chromosome.

1952 Karl Maramorosch demonstrates that some viruses can multiply in both plants and insects. This work leads to new questions about the origins of viruses.

1952 Lederberg and Ester Lederberg develop the replica plating method that allows for the rapid screening of large numbers of genetic markers. They use the technique to demonstrate that resistance to antibacterial agents such as antibiotics and viruses is not induced by the presence of the antibacterial agent.

1952 Renato Dulbecco develops a practical method for studying animal viruses in cell cultures. His so-called plaque method is comparable to that used in studies of bacterial viruses, and the method proves to be important in genetic studies of viruses. These methods are described in his paper "Production of Plaques in Monolayer Tissue Cultures by Single Particles of an Animal Virus."

1952 William Hayes isolates a strain of *E. coli* that produces recombinants thousands of times more frequently than previously observed. The new strain of K12 is named Hfr (high-frequency recombination) Hayes.

1953 James D. Watson and Francis H. C. Crick publish two landmark papers in the journal *Nature*, "Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid" and "Genetical implications of the structure of deoxyribonucleic acid." Watson and Crick propose a double helical model for DNA and call attention to the genetic implications of their model. Their model is based, in part, on the x-ray crystallographic work of Rosalind Franklin and the biochemical work of Erwin Chargaff. Their model explains how the genetic material is transmitted.

1953 Jonas Salk begins testing a polio vaccine comprised of a mixture of killed viruses.

1954 Seymour Benzer deduces the fine structure of the rII region of the bacteriophage T4 of *Escherichia coli*, and coins the terms cistron, recon, and muton.

1955 François Jacob and Elie L. Wollman determine the mechanism of the transmission of genetic information during bacterial mating. Jacob and Wollman use a blender to interrupt the mating process and then determine the sequence of genetic transfer between bacterial cells.

1955 Fred L. Schaffer and Carlton E. Schwerdt report on their successful crystallization of the polio virus. Their achievement is the first successful crystallization of an animal virus.

1955 Heinz Fraenkel-Conrat and Robley C. Williams prove that tobacco mosaic virus can be reconstituted from its nucleic acid and protein subunits. The reconstituted particles exhibit normal morphology and infectivity.

1956 Alfred Gierer and Gerhard Schramm demonstrate that naked RNA from tobacco mosaic virus is infectious. Subsequently, infectious RNA preparations are obtained for certain animal viruses.

1956 Arthur Kornberg demonstrates the existence of DNA polymerase in *Escherichia coli*.

1956 Joe Hin Tijo and Albert Levan prove that the number of chromosomes in a human cell is 46, not 48, as had been argued since the early 1920s.

1957 Alick Isaacs and Jean Lindenmann discover and publish their pioneering report on interferon, a protein produced by interaction between a virus and an infected cell that can interfere with the multiplication of viruses.

1957 François Jacob and Elie L. Wollman demonstrate that the single linkage group of *Escherichia coli* is circular; they suggest that the different linkage groups found in different Hfr strains are the results of different insertion points of a factor in the circular linkage group, which determines the rupture of the circle.

1957 Francis Crick proposes that during protein formation each amino acid is carried to the template by an adapter molecule containing nucleotides and that the adapter is the part that actually fits on the RNA template. Later research demonstrates the existence of transfer RNA.

1957 The World Health Organization advances the oral polio vaccine developed by Albert Sabin as a safer alternative to the Salk vaccine.

1958 Frederick Sanger is awarded the Nobel Prize in chemistry for his work on the structure of proteins, especially for determining the primary sequence of insulin.

1958 George W. Beadle, Edward L. Tatum, and Joshua Lederberg are awarded the Nobel Prize in Medicine or Physiology. Beadle and Tatum are honored for their work in *Neurospora* that led to the one gene–one enzyme theory. Lederberg is honored for discoveries concerning genetic recombination and the organization of the genetic material of bacteria.

1958 Matthew Meselson and Frank W. Stahl publish their landmark paper "The replication of DNA in Escherichia coli," which demonstrates that the replication of DNA follows the semiconservative model.

1959 Arthur Kornberg and Severo Ochoa are awarded the Nobel Prize in Medicine or Physiology for their discovery of enzymes that produce artificial DNA and RNA.

1959 Robert L. Sinsheimer reports that bacteriophage ØX174, which infects *Escherichia coli*, contains a single-stranded DNA molecule, rather than the expected double stranded DNA. This provides the first example of a single-stranded DNA genome.

1959 Sydney Brenner and Robert W. Horne publish a paper entitled The two researchers develop a method for studying the architecture of viruses at the molecular level using the electron microscope.

1959 English biochemist Rodney Porter begins studies that lead to the discovery of the structure of antibodies. Porter receives the 1972 Nobel Prize in Physiology or Medicine for this research.

1961 François Jacob and Jacques Monod publish *Genetic regulatory mechanisms in the synthesis of proteins*, a paper that describes the role of messenger RNA and proposes the operon theory as the mechanism of genetic control of protein synthesis.

1961 Francis Crick, Sydney Brenner, and others propose that a molecule called transfer RNA uses a three-base code in the manufacture of proteins.

1961 Marshall Warren Nirenberg synthesizes a polypeptide using an artificial messenger RNA (a synthetic RNA containing only the base uracil) in a cell-free protein-synthesizing system. The resulting polypeptide contains only the amino acid phenylalanine, indicating that UUU was the codon for phenylalanine. This important step in deciphering the genetic code is described in the landmark paper by Nirenberg and J. Heinrich Matthaei, "The Dependence of Cell-Free Synthesis in E. coli upon Naturally Occurring or Synthetic Polyribonucleotides." This work establishes the messenger concept and a system that could be used to work out the relationship between the sequence of nucleotides in the genetic material and amino acids in the gene product.

1961 French pathologist Jacques Miller discovers the role of the thymus in cellular immunity.

1961 Noel Warner establishes the physiological distinction between the cellular and humoral immune responses.

1962 James D. Watson, Francis Crick, and Maurice Wilkins are awarded the Nobel Prize in Medicine or Physiology for their work in elucidating the structure of DNA.

1963 Ruth Sager discovers DNA in chloroplasts. Boris Ephrussi discovers DNA in mitochondria.

1964 Barbara Bain publishes a classic account of her work on the mixed leukocyte culture (MLC) system that is critical in determining donor-recipient matches for organ or bone marrow transplantation. Bain shows that the MLC phenomenon is caused by complex genetic differences between individuals.

1965 François Jacob, André Lwoff, and Jacques Monod are awarded the Nobel Prize in Medicine or Physiology for their discoveries concerning genetic control of enzymes and virus synthesis.

1966 Bruce Ames develops a test to screen for compounds that cause mutations, including those that are cancer causing. The so-called Ames test utilizes the bacterium *Salmonella typhimurium*.

1966 Marshall Nirenberg and Har Gobind Khorana lead teams that decipher the genetic code. All of the 64 possible triplet combinations of the four bases (the codons) and their associated amino acids are determined and described.

1967 Charles T. Caskey, Richard E. Marshall, and Marshall Warren Nirenberg suggest that there is a universal genetic code shared by all life forms.

1967 Charles Yanofsky demonstrates that the sequence of codons in a gene determines the sequence of amino acids in a protein.

1967 Thomas Brock discovers the heat-loving bacterium *Thermus aquaticus* from a hot spring in Yellowstone National Park. The bacterium yields the enzyme that becomes the basis of the DNA polymerase reaction.

1968 Lynne Margulis proposes that mitochondria and chloroplasts in eukaryotic cells originated from bacterial symbiosis.

1968 Mark Steven Ptashne and Walter Gilbert independently identify the bacteriophage genes that are the repressors of the lac operon.

1968 Werner Arber discovers that bacteria defend themselves against viruses by producing DNA-cutting enzymes. These enzymes quickly become important tools for molecular biologists.

1969 Julius Adler discovers protein receptors in bacteria that function in the detection of chemical attractants and repellents. The so-called chemoreceptors are critical for the directed movement of bacteria that comes to be known as chemotaxis.

1969 Max Delbrück, Alfred D. Hershey, and Salvador E. Luria are awarded the Nobel Prize in Medicine or Physiology for their discoveries concerning the replication mechanism and the genetic structure of viruses.

1969 Stanford Moore and William H. Stein determine the sequence of the 124-amino-acid chain of the enzyme ribonuclease.

1970 Hamilton Smith and Kent Wilcox isolate the first restriction enzyme, HindII, an enzyme that cuts DNA molecules at specific recognition sites.

1970 Har Gobind Khorana announce the synthesis of the first wholly artificial gene. Khorana and his coworkers synthesize the gene that codes for alanine transfer RNA in yeast.

1970 Howard Martin Temin and David Baltimore independently discover reverse transcriptase in viruses. Reverse transcriptase is an enzyme that catalyzes the transcription of RNA into DNA.

1971 Christian B. Anfinsen, Stanford Moore, and William H. Stein are awarded the Nobel Prize in chemistry. Anfinsen is cited for his work on ribonuclease, especially concerning the connection between the amino acid sequence and the biologically active conformation. Moore and Stein are cited for their contribution to the understanding of the connection between chemical structure and catalytic activity of the active center of the ribonuclease molecule.

1972 Paul Berg and Herbert Boyer produce the first recombinant DNA molecules.

1972 Recombinant technology emerges as one of the most powerful techniques of molecular biology. Scientists are able to splice together pieces of DNA to form recombinant genes. As the potential uses—therapeutic and industrial—became increasingly clear, scientists and venture capitalists establish biotechnology companies.

1973 Annie Chang and Stanley Cohen show that a recombinant DNA molecule can be maintained and replicated in *Escherichia coli*.

1973 Concerns about the possible hazards posed by recombinant DNA technologies, especially work with tumor viruses, leads to the establishment of a meeting at Asilomar, California. The proceedings of this meeting are subsequently published by the Cold Spring Harbor Laboratory as a book entitled *Biohazards in Biological Research*.

1973 Herbert Wayne Boyer and Stanley H. Cohen create recombinant genes by cutting DNA molecules with restriction enzymes. These experiments mark the beginning of genetic engineering.

1973 Joseph Sambrook and coworkers refine DNA electrophoresis by using agarose gel and staining with ethidium bromide.

1974 Peter Doherty and Rolf Zinkernagel discover the basis of immune determination of self and non-self.

1975 César Milstein and George Kohler create monoclonal antibodies.

1975 David Baltimore, Renato Dulbecco, and Howard Temin share the Nobel Prize in Medicine or Physiology for their discoveries concerning the interaction between tumor viruses and the genetic material of the cell and the discovery of reverse transcriptase.

1976 First outbreak of Ebola virus observed in Zaire. There are more than 300 cases and a 90% death rate.

1976 Michael J. Bishop, Harold Elliot Varmus, and coworkers obtain definitive evidence that confirms the oncogene hypothesis. They discover that normal genes can malfunction and cause cells to become cancerous.

1977 Carl R. Woese and George E. Fox publish an account of the discovery of a third major branch of living beings, the Archaea. Woese suggests that an rRNA database could be used to generate phylogenetic trees.

1977 The last reported smallpox case is recorded. Ultimately, the World Health Organization (WHO) declares the disease eradicated.

1977 Frederick Sanger develops the chain termination (dideoxy) method for sequencing DNA, and uses the method to sequence the genome of a microorganism.

1977 Holger Jannasch demonstrates that heat-loving bacteria found at hydrothermal vents are the basis of an ecosystem that exists in the absence of light.

1977 Philip Allen Sharp and Richard John Roberts independently discover that the DNA making up a particular gene could be present in the genome as several separate segments. Although both Roberts and Sharp use a common cold–causing virus, called adenovirus, as their model system, researchers later find "split genes" in higher organisms, including humans. Sharp and Roberts are subsequently awarded the Nobel Prize in Medicine or Physiology in 1993 for the discovery of split genes.

1980 Paul Berg, Walter Gilbert, and Frederick Sanger share the Nobel Prize in Chemistry. Berg is honored for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA. Gilbert and Sanger are honored for their contributions to the sequencing of nucleic acids. This is Sanger's second Nobel Prize.

1980 Researchers successfully introduce a human gene, which codes for the protein interferon, into a bacterium.

1980 The United States Supreme Court rules that a living organism developed by General Electric (a microbe used to clean up an oil spill) can be patented.

1981 Karl Illmensee clones baby mice.

1982 The United States Food and Drug Administration approves the first genetically engineered drug, a form of human insulin produced by bacteria.

1983 O157:H7 is identified as a human pathogen.

1983 Andrew W. Murray and Jack William Szostak create the first artificial chromosome.

1983 Luc Montagnier and Robert Gallo discover the human immunodeficiency virus that is believed to cause acquired immunodeficiency syndrome (AIDS).

1984 Steen A. Willadsen successfully clones a sheep.

1984 The United States Department of Energy (DOE), Office of Health and Environmental Research, U.S. Department of Energy (OHER, now Office of Biological and Environmental Research), and the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) cosponsor the Alta, Utah, conference highlighting the growing role of recombinant DNA technologies. OTA incorporates the proceedings of the meeting into a report acknowledging the value of deciphering the human genome.

1985 Alec Jeffreys develops "genetic fingerprinting," a method of using DNA polymorphisms (unique sequences of DNA) to identify individuals. The method, which is subsequently used in paternity, immigration, and murder cases, is generally referred to as "DNA fingerprinting."

1985 Elizabeth Blackburn and Carol Greider discover the enzyme telomerase, an unusual RNA-containing DNA polymerase that can add to the telomeres (specialized structures found at the ends of chromosomal DNA). Telomeres appear to protect the integrity of the chromosome. Most normal somatic cells lack telomerase, but cancer cells have telomerase activity, which might explain their ability to multiply indefinitely.

1985 Kary Mullis, who was working at Cetus Corporation, develops the polymerase chain reaction (PCR), a new method of amplifying DNA. This technique quickly becomes one of the most powerful tools of molecular biology. Cetus patents PCR and sells the patent to Hoffman-LaRoche, Inc. in 1991.

1985 Japanese molecular biologist Susuma Tonegawa discovers the genes that code for immunoglobulins. He receives the 1986 Nobel Prize in Physiology or Medicine for this discovery.

1985 American molecular biologist and physician Leroy Hood leads a team that discovers the genes that code for the T cell receptor.

1986 The United States Food and Drug Administration approves the first genetically engineered human vaccine for hepatitis B.

1987 Maynard Olson creates and names yeast artificial chromosomes (YACs), which provided a technique to clone long segments of DNA.

1987 The United States Congress charters a Department of Energy advisory committee, The Health and Environmental Research Advisory Committee (HERAC), which recommends a 15–year, multidis-

ciplinary, scientific, and technological undertaking to map and sequence the human genome. DOE designates multidisciplinary human genome centers. National Institute of General Medical Sciences at the National Institutes of Health (NIH NIGMS) begin funding genome projects.

1988 The Human Genome Organization (HUGO) is established by scientists in order to coordinate international efforts to sequence the human genome.

1989 Cells from one embryo are used to produce seven cloned calves.

1989 James D. Watson is appointed head of the National Center for Human Genome Research. The agency is created to oversee the $3 billion budgeted for the American plan to map and sequence the entire human DNA by 2005.

1989 Sidney Altman and Thomas R. Cech are awarded the Nobel Prize in chemistry for their discovery of ribozymes (RNA molecules with catalytic activity). Cech proves that RNA could function as a biocatalyst as well as an information carrier.

1990 Michael R. Blaese and French W. Anderson conduct the first gene replacement therapy experiment on a four-year-old girl with adenosine deaminase (ADA) deficiency, an immune-system disorder. T cells from the patient are isolated and exposed to retroviruses containing an RNA copy of a normal ADA gene. The treated cells are returned to her body where they help restore some degree of function to her immune system.

1990 Research and development begins for the efficient production of more stable, large-insert bacterial artificial chromosomes (BACs).

1991 The Genome Database, a human chromosome mapping data repository, is established.

1992 Craig Venter establishes The Institute for Genomic Research (TIGR) in Rockville, Maryland. TIGR later sequences the genome of *Haemophilus influenzae* and many other bacterial genomes.

1992 Francis Collins replaces James Watson as head of the National Center for Human Genome Research at the National Institutes of Health. Watson had clashed with Craig Venter, then at NIH, over the patenting of DNA fragments known as "expressed sequence tags."

1992 Guidelines for data release and resource sharing related to the Human Genome Project are announced by the United States Department of Energy and National Institutes of Health.

1993 Hanta virus emerged in the United States in a 1993 outbreak on a "Four Corners" area (the juncture of Utah, Colorado, New Mexico, Arizona) Native American reservation. The resulting Hanta pulmonary syndrome (HPS) had a 43% mortality rate.

1993 French Gépnéthon makes mega-YACs available to the genome community.

1993 George Washington University researchers clone human embryos and nurture them in a Petri dish for several days. The project provokes protests from many ethicists, politicians, and other critics of genetic engineering.

1994 DOE announce the establishment of the Microbial Genome Project as a spin-off of the Human Genome Project.

1994 Geneticists determine that DNA-repair enzymes perform several vital functions, including preserving genetic information and protecting the cell from cancer.

1994 The 5-year goal for genetic-mapping is achieved one year ahead of schedule.

1994 The Human Genome Project Information Web site is made available to researchers and the public.

1995 Peter Funch and Reinhardt Moberg Kristensen create a new phylum, Cycliophora, for a novel invertebrate called *Symbion pandora*, which is found living in the mouths of Norwegian lobsters.

1995 Researchers at Duke University Medical Center report that they have transplanted hearts from genetically altered pigs into baboons. All three transgenic pig hearts survive at least a few hours, suggesting that xenotransplants (cross-species organ transplantation) might be possible.

1995 The genome of the bacterium *Haemophilus influenzae* is sequenced.

1995 The sequence of *Mycoplasma genitalium* is completed. *Mycoplasma genitalium*, regarded as the smallest known bacterium, is considered a model of the minimum number of genes needed for independent existence.

1996 International participants in the genome project meet in Bermuda and agree to formalize the conditions of data access. The agreement, known as the "Bermuda Principles," calls for the release of sequence data into public databases within 24 hours.

1996 Scientists report further evidence that individuals with two mutant copies of the CC-CLR-5 gene are generally resistant to HIV infection.

1996 The sequence of the *Methanococcus jannaschii* genome provides further evidence of the existence of third major branch of life on earth.

1996 William R. Bishai and co-workers report that SigF, a gene in the tuberculosis bacterium, enables the bacterium to enter a dormant stage.

1997 Ian Wilmut of the Roslin Institute in Edinburgh, Scotland, announces the birth of a lamb called Dolly, the first mammal cloned from an adult cell (a cell in a pregnant ewe's mammary gland).

1997 The DNA sequence of *Escherichia coli* is completed.

1997 The National Center for Human Genome Research (NCHGR) at the National Institutes of Health becomes the National Human Genome Research Institute (NHGRI).

1997 William Jacobs and Barry Bloom create a biological entity that combines the characteristics of a bacterial virus and a plasmid (a DNA structure that functions and replicates independently of the chromosomes). This entity is capable of triggering mutations in *Mycobacterium tuberculosis.*

1998 Craig Venter forms a company (later named Celera), and predicts that the company would decode the entire human genome within three years. Celera plans to use a "whole genome shotgun" method, which would assemble the genome without using maps. Venter says that his company would not follow the Bermuda principles concerning data release.

1998 DOE funds bacterial artificial chromosome and sequencing projects.

1998 Dolly, the first cloned sheep, gives birth to a lamb that had been conceived by a natural mating with a Welsh Mountain ram. Researches said the birth of Bonnie proved that Dolly was a fully normal and healthy animal.

1998 Immunologist Ellen Heber-Katz, researcher at the Wistar Institute in Philadelphia, reports than a strain of laboratory mice can regenerate tissue in their ears, closing holes that scientists had created for identification purposes. This discovery reopens the discussion on possible regeneration in humans.

1998 The genome of the *Mycobacterium tuberculosis* bacterium is sequenced.

1998 Two research teams succeed in growing embryonic stem cells.

1999 The public genome project responds to Craig Venter's challenge with plans to produce a draft genome sequence by 2000. Most of the sequencing is done in five centers, known as the "G5": the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts; the Sanger Centre near Cambridge, United Kingdom; Baylor College of Medicine in Houston, Texas; Washington University in St. Louis, Missouri; the DOE's Joint Genome Institute (JGI) in Walnut Creek, California.

2000 On June 26, 2000, leaders of the public genome project and Celera announce the completion of a working draft of the entire human genome sequence. Ari Patrinos of the DOE helps mediate disputes between the two groups so that a fairly amicable joint announcement could be presented at the White House in Washington, DC.

2001 The complete draft sequence of the human genome is published. The public sequence data is published in the British journal *Nature* and the Celera sequence is published in the American journal *Science*.

2001 United States President George Bush announces the United States will allow and support limited forms of stem cell growth and research.

2001 In the aftermath of the September 11 terrorist attacks on the United States, a number of deaths result from the deliberate release of the bacterial agent of anthrax.

2001 Advanced Cell Technology announces that its researchers have created cloned human embryos that grew to the six-cell stage.

2002 In the aftermath of the September 11, 2001 terrorist attacks on the United States, the United States Government dramatically increases funding to programs concerned with research on microorganisms and other agents that could potentially be used in bioterrorist attacks.

2002 Traces of biological and chemical weapon agents are found in Uzbekistan on a military base used by U.S. troops fighting in Afghanistan. Early analysis dates and attributes the source of the contamination to former Soviet Union biological and chemical weapons programs that utilized the base.

2002 The planned destruction of stocks of smallpox causing Variola virus at the two remaining depositories in the US and Russia is delayed over fears that large scale production of vaccine might be needed in the event of a bioterrorist action.

General Index

A

Abbé, Ernst, **1:1,** 2:388
Abel, John Jacob, 2:650
Abraham, E.P., 2:653
Accutane, 1:2
Acid-loving bacteria, 1:211
Acidity, pH, 2:433
aCL. *See* Anticardiolipin antibodies
Acne, microbial basis of, **1:1–2,** 1:*2*
    See also Microbial flora of the skin
Acquired immunity, 2:361–362
Acquired resistance, 1:47
Acrasiomycota, 2:518
Acridine orange, **1:2–3,** 1:24
Actinomyces, **1:3,** 1:132
Actinomyces infection, 1:3
Actinomycosis, 1:3
Activated sludge process, 1:218
Active immunity, 1:288–290
Active transport, 1:109
Acyclovir, 1:116, 1:184, 2:572, 2:573
ADA deficiency, 1:297
Adams, George, 2:646
Adenosine triphosphate (ATP), 2:392, 2:484–485
Adenoviruses, **1:3–4,** 1:*4,* 2:579, 2:*580,* 2:581, 2:584
Adickes, Franz, 1:178
Adjuvant, **1:4–5,** 2:567–568
Adjuvant therapy, 1:116
Adler, Julius, 2:657
Aedes aegypti, 1:153
Aedes albopictus, 1:153
Aerobes, **1:5**
    activated sludge process, 1:218
    Azotobacter, 1:41
    electron transport system, 1:182
    photosynthetic microorganisms, 2:437
    wastewater treatment, 1:218
Aerobic respiration, 2:484–485
Aerolysin, 1:189
Aeromonas hydrophila, exotoxin, 1:189
Aflatoxin, 2:394, 2:395
AFM. *See* Atomic force microscope
African sleeping sickness, 2:462
African swine fever, 2:536
African trypanosomiasis, 2:462
Agammaglobulinemia, 1:293, 1:301
Agar and agarose, **1:5–7,** 1:*6,* 1:23, 1:255, 2:*473*
Agar diffusion, **1:7,** 1:*7*
Agar diffusion assay, 1:7
Agaropectin, 1:6
Agarose, 1:6
Agrobacterium, 1:354
Agrobacterium tumefaciens, 2:442
AIDS, **1:7–10,** 1:296, 2:512
    animal models of infection, 1:19
    antiviral drugs, 1:33
    Elion, Gertrude Belle, 1:183–185
    epidemics, 1:196–198
    human T-cell leukemia virus (HTLV), 1:281
    Kaposi's sarcoma, 1:267, 1:314
    mycobacteria, atypical, 2:407
    mycoplasma infections, 2:408
    pneumonia with, 2:445
    as public health issue, 1:19–22, 2:467
    recent advances in, 2:401, 2:486, 2:493–494
    retroviruses, 2:486–487
    seroconversion, 2:508
    treatment for, 2:487
    vaccine, 1:9, 2:513, 2:570–571
    See also Human immunodeficiency virus
AIDS, recent advances in research and treatment, 2:401
    retroviruses, 2:486–487
    RNA tumor viruses, 2:493–494
Alcmaeon, 2:643
Alcohol, as disinfectant, 1:159
Alcoholic beverages, fermentation, 1:217
Aldehyde compounds, as disinfectant, 1:159
Alexander, Hattie Elizabeth, **1:10**
Alexandrium, 2:481
Alexin, 1:287
Algae, 2:387, 2:460

aerobes, 1:5
blue-green algae, 1:82–83, 1:119, 1:120, 1:154, 1:203, 1:228, 1:235, 2:436
brown algae, 1:6, 1:323–324, 2:421, 2:460
cryoprotection, 1:141–142
diatoms, 1:154–155
dinoflagellates, 1:156
green algae, 2:460
lichens, 1:348–349, 2:407, 2:411
Phaeophyta, 2:421, 2:460
photosynthesis, 2:436
red algae, 2:460, 2:462, 2:488
red-brownish algae, 2:469
red tide, 1:156–157, 2:460, 2:481–482
Rhodophyta, 2:488
snow blooms, 2:522
Xanthophyta, 2:605–606
yellow-green algae, 2:605–606
See also Photosynthetic microorganisms
Alkaline-loving bacteria, 1:211
Alkalinity, pH, 2:433
Allergic rhinitis, 1:10
Allergies, **1:10–11,** 1:*11*
anaphylaxis, 1:17
to antiseptic, 1:31
hives, 1:*311*
Allopurinol, 1:184
Allotypes, **1:315–316**
Alper, Tikvah, 1:92, 2:464
Alpha globulins, 1:249
Alpha hemolysis, 1:80
Alpha radiation, 2:479
Altman, Sidney, 2:659
Altmann, Richard, 2:650
Alzheimer's disease, metals, 1:92
Amantadine, 1:33, 1:116
Amber, fossils in, 1:228
Amebiasis, 1:11
Amebic dysentery, **1:11–12,** 1:169, 2:423
Entamoeba histolytica, 1:169, 1:186–187
Giardia and giardiasis, 1:248–249, 2:462
American Type Culture Collection (ATCC), 1:*12,* **1:12–13,** 2:383
Ames, Bruce N., **1:13–14,** 2:657
Amino acid chemistry, **1:14–16,** 1:*15*
genetic code, 1:238
Murchison meteorite, 2:403
protein synthesis, 2:454–455
See also Proteins and enzymes
Ammonia, 2:410
Ammonification, 2:411
Ammonium, 2:410
Amnesic shellfish poisoning, 2:482
Amoeba, 1:155, 2:459, 2:462
Amoebic dysentery. *See* Amebic dysentery
Amphipods, 2:616
ANA. *See* Antinuclear antibodies
Anaerobes and anaerobic infection, **1:16–17**
Actinomyces, 1:3
Enterobacteriaceae, 1:187–188
extraterrestrial microbiology, 1:209–211
methane oxidizing and producing bacteria, 2:378–379
photosynthetic bacteria, 2:436
tetanus and tetanus immunization, 2:543
Anaphase, 1:103, 1:107, 1:244
Anaphase I, 1:105
Anaphylactic shock, 1:17
Anaphylaxis, **1:17,** 1:287
Anaplerosis, 2:377
Anderson, French W., 2:659
Anderson, Thomas F., 2:653
Andes virus, 1:259
Andrewes, Christopher H., 2:653
Anfinsen, Christian B., 2:657
Anger, Hal, 2:479
Animal models of infection, 1:*18,* **1:18–19**
foot-and-mouth disease, 1:227–228, 1:354
hepadnaviruses, 1:264
Koch's postulates, 1:247, 1:274, 1:327, 1:328, 1:353
swine fever, 2:536–537
See also Zoonoses
Animals
See also Animal models of infection; Parasites; Veterinary microbiology; Zoonoses
Animals, cloning, 1:124
Anopheles mosquito, 2:443–444
Anostraca, 2:616
Anthrax, **1:19–22,** 1:*20*
antibiotics, 1:21, 2:467, 2:*467*
biological warfare, 1:70, 1:71
diagnosis of, 1:241
enterotoxin and exotoxin, **1:189**
inhalation anthrax, 1:70, 1:76
Koch, Robert, 1:19, 1:247, 1:326
terrorist use of as a biological weapon, 1:19–22, 1:241
Anthrax, terrorist use as a biological weapon, **1:19–22,** 1:*22,* 1:241
Biological Weapons Convention (BWC), 1:71–72, 1:78
transmission of pathogens, 2:553
Anti-adhesion methods, **1:22–23,** 1:67–68
Anti-cancer drugs. *See* Chemotherapy
Anti-mitotic signals, 1:104, 1:106, 1:243
Antibacterial agents
See also Antibiotics
Antibacterial agents, acne and, 1:2
Antibiotic resistance, 1:25, 1:309, 2:428
bacterial adaptation, 1:47
biofilms, 1:68
nosocomial infections, 2:412
staphylococci, 2:412
See also Antibiotic resistance, tests for
Antibiotic resistance, tests for, **1:23–24**
agar diffusion, 1:7
indicator species, 1:308
rare genotype advantage, 2:480
seroconversion, 2:508
sulfa drugs, 2:535
See also Antibiotic resistance
Antibiotics, **1:24–25,** 1:46–47
anaerobes, 1:17
anthrax, 1:21, 2:467, 2:*467*
bacteriocidal, bacteriostatic, 1:54–55
Bayer, Manfred E., 1:57–58
bubonic plague, 1:93, 1:95
chemotherapy, 1:116–117
chlamydial pneumonia, 1:118
diphtheria, 1:158
by fermentation, 1:218

Fleming, Alexander, 1:218–219
gonorrhea, 1:251
meningitis, 2:374
Pasteurella, 2:426
penicillin, 1:25, 2:427–429
pertussis, 2:430
pneumonia, 2:445
rickettsial diseases, 2:492
Streptococcus, 2:533, 2:535
streptomycin, 1:116, 2:588
sulfa drugs, 2:535
See also History of development of antibiotics
Antibody and antigen, **1:25–30,** 1:*28,* 1:302–303, 1:304
adjuvant, 1:4–5
allergies, 1:10–11
anaphylaxis, 1:17
antibody formation and kinetics, 1:29
antigenic mimicry, 1:30–31
B cells, 1:43
biochemical and molecular reactions, 1:25–26
histocompatibility, 1:271
human leukocyte antigen, 1:280
immune complex test, 1:285–286
immune synapse, 1:286–287
immune system, 1:287–288
interferons, 1:313–314
isotypes and allotypes, 1:315–316
major histocompatibility complex (MHC), 2:361–363
monoclonal antibodies, 1:28, 1:29–30, 1:304, 2:434–435
phagocyte and phagocytosis, 2:434–435
protein crystallography, 2:452
Rh and Rh incompatibility, 2:487–488
seroconversion, 2:508
streptococcal antibody tests, 2:533
transplantation genetics and immunology, 2:553–554
See also Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Laboratory techniques in immunology
Antibody-antigen, biochemical and molecular reactions, **1:25–26**
phagocyte and phagocytosis, 2:434–435
protein crystallography, 2:452
seroconversion, 2:508
Antibody formation and kinetics, **1:29**
B cells, 1:43
immune synapse, 1:286–287
phagocyte and phagocytosis, 2:434–435
Rh and Rh incompatibility, 2:487–488
seroconversion, 2:508
streptococcal antibody tests, 2:533
typhoid fever, 2:558–560
vaccine, 2:568–572
Antibody, monoclonal, 1:28, **1:29–30,** 1:304, 1:334, 2:434–435
Anticardiolipin antibodies (aCL), 2:483
Antideoxyribonuclease-B titer (anti-Dnase-B), 2:533
Antigenic mimicry, **1:30–31,** 2:434–435
Antigens. *See* Antibody and antigen
Antihistamines, allergies, 1:11
Antinuclear antibodies (ANA), 2:483
Antiphospholipid antibody (APL), 1:38
Antiporter, 2:451
Antiretroviral drugs, 1:33
Antiseptics, **1:31–32,** 1:283–284
Antiserum and antitoxin, **1:32,** 1:58–59, 1:158
Antistreptolysin O titer (ASO titer), 2:533
Antitoxin. *See* Antiserum and antitoxin
Antiviral drugs, **1:33**
antiserum and antitoxin, 1:32
influenza, 1:313
Antonine plague, 2:644
Apex, 1:3
Apicomplexa, 2:526
APL. *See* Antiphospholipid antibody
Appert, Nicolas François, **1:33–34**
Aquaspirillum magnetotacticum, 2:360, 2:526
Arachnides, 2:423
Arber, Werner, 2:657
Archaea, **1:34,** 1:51
mesophilic bacteria, 2:376
metabolism, 2:377
photosynthetic microorganisms, 2:437
Archaebacteria, 1:88, 2:450
Arenavirus, **1:34–35,** 1:263
Argentine hemorrhagic fever, 1:34, 1:263
Aristotle, 2:643
Armillaria ostoyae, **1:35**
Artenstein, Malcolm Stewart, 2:424
Arthritis, mycoplasma and, 2:408
Arthropods, as carriers of disease, 2:423
Ascomycetes, 1:348, 2:407
Ascomycotina, 2:394
Asexual generation and reproduction, **1:35–36,** 1:*36,* 2:527
Asfivirus, 2:536
Asian flu, 1:196–197
Asilomar Conference, **1:36,** 1:60–62
ASO titer. *See* Antistreptolysin O titer
Aspergillus flavus, 2:394, 2:395
Aspergillus nidulans, 1:230, 2:404
Aspergillus terreus, 2:395
Ataxia telangiectasia, 1:297
ATCC. *See* American Type Culture Collection
Athlete's foot, 2:517
Atomic force microscope (AFM), **1:36–37**
ATP. *See* Adenosine triphosphate
ATP synthase, 1:182
Attractants and repellents, **1:37**
Audureau, Alice, 2:398
Aureomycin, 1:116
Autoclave, 1:160
Autoclaving, 2:530–531
Autoimmunity and autoimmune diseases
antigenic mimicry, 1:30–31
cytokines, 1:104, 1:145
electrophoresis, 1:182–183
immunogenetics, 1:299–300
immunosuppressant drugs, 1:306–307
Lichen planus, 1:348
reproductive immunology, 2:483–484
Autoimmunity and autoimmune disorders, **1:38**
Autologous banking, **1:38–39**
Automatic dispensing pipette, 2:439
Autoradiography, 2:479
Autotrophic bacteria, **1:39,** 1:115, 1:255
chlorophyll, 1:119
chloroplast, 1:120
Eubacteria, 1:203

photosynthetic microorganisms, 2:437
xanthophylls, 2:605
Avery, Oswald Theodore, **1:39–41,** 1:114, 1:167, 1:274, 1:337, 2:359, 2:367, 2:382, 2:654
Avihepadnavirus, 1:264
Axial filaments, 1:48, 1:52, 2:525
Azidothymidine (AZT), 1:8, 1:116, 1:183
Azotobacter, **1:41**
AZT, 1:8, 1:116, 1:183

B

B cells (B lymphocytes), **1:43,** 1:288, 1:291, 1:293, 2:539
Epstein-Barr virus, 1:201
immune system, 1:287–288
B lymphocyte deficiency, 1:293
Babesia, 2:464, 2:526
Babesiosis, 1:82
BAC. *See* Bacterial artificial chromosome
Bacillariophyta, 2:460
Bacillary dysentery, 1:168, 2:514
Bacillus anthracis
anthrax, 1:19–21, 1:*20*
biological warfare, 1:70
surface layers, 1:53
Bacillus Calmette-Guerin, 1:286
Bacillus stearothermophilus, 1:160, 2:405
Bacillus subtilis
colony, 1:129
sporulation, 2:527
thermal death, 2:546
Bacillus thuringiensis, **1:43–44,** 1:*44*
Bacon, Roger, 2:644
Bacteremia, 1:44, 1:45
Bacteremic, **1:44–45,** 2:435
Bacteria
acid-loving bacteria, 1:211
Actinomyces, 1:3
adaptation, 1:46–47
aerobes, 1:5
alkaline-loving bacteria, 1:211
anaerobes and anaerobic infection, **1:16–17**
appendages, 1:47–48, 1:52, 1:67
asexual generation and reproduction, 1:35–36
attractants and repellents, 1:37
autotrophic bacteria, 1:39, 1:115
Azotobacter, 1:41
bacterial kingdoms, 1:51
bioluminescence, 1:72–73, 1:354
Caulobacter crescentus, 1:101
chemoautotrophic and chemolithotrophic bacteria, 1:115–116
cloroxybacteria, 2:436
colony and colony formation, 1:129–130
contamination, 1:135–136
coryneform bacteria, 1:136–137, 1:157
Coulter counter, 1:137–138
cryoprotection, 1:141–142
cyanobacteria, 1:82–83
DNA, 1:161
electron transport system, 1:182
Enterobacteriaceae, 1:187–188
environmental contamination, 1:136–137
episomes, plasmids, insertion sequences, and transposons, 1:200
Eubacteria, 1:203
evolutionary origin, 1:208–209
extremophiles, 1:211–212
fossilization of bacteria, 1:228
Francisella tularensis, 2:557
germ theory of disease, 1:28, 1:246–247, 1:273
gliding bacteria, 1:52, 1:249
growth and division, 1:49–51
Haemophilus, 1:257
halophilic bacteria, 1:211
indicator species, 1:308
invasiveness and intracellular infection, 1:315
"iron" bacteria, 1:115, 2:514
lactic acid bacteria, 1:336
Lactobacillus, 1:336–337
luminescent bacteria, 1:354
magnetotactic bacteria, 2:360–361
mesophilic bacteria, 1:50, 1:276
methane oxidizing and producing bacteria, 1:101, 2:378–379
microbial flora of the oral cavity, dental caries, 2:379–380
microbial flora of the skin, 2:380
microbial flora of the stomach and gastrointestinal tract, 2:380–381
movement of, 1:52
nitrogen-fixing bacteria, 2:410
nosocomial infection, 2:411–412
Pasteurella, 2:426
photosynthetic, 2:436, 2:437
plankton and planktonic bacteria, 2:440–441
plaque, 1:17, 1:67, 2:387, 2:442
probiotics, 2:450
protein export, 2:453–454
protoplasts and spheroplasts, 2:462
Pseudomonas, 2:465–466
psychrophilic bacteria, 2:466
purple non-sulfur bacteria, 2:436
radiation-resistant bacteria, 2:478–479, 2:532
Salmonella, 2:503–504
sensitivity to temperature and pH ranges, 2:404, 2:433
sheathed bacteria, 2:514
Shigella, 2:514–515
spirochetes, 1:48, 1:52, 2:384, 2:385, 2:525–526
staphylococci and staphylococci infections, **2:529–530**
streptococci and streptococcal infections, **2:533–535**
surface layers, 1:53
thermophilic bacteria, 1:133, 1:211
transposons, 1:126, 1:200, 2:554
ultrastructure, 1:53–54
viable but nonculturable bacteria, 2:577
Bacteria and bacterial infection, **1:45–46,** 1:*46*
See also Bacteria; Bacterial infection
Bacterial adaptation, **1:46–47**
antibiotic resistance, 1:47
biofilms, 1:67–68
heat shock response, 1:261
luminescent bacteria, 1:354
sensitivity to temperature and pH ranges, 2:404–405, 2:433
sporulation, 2:527
viable but nonculturable bacteria, 2:577
Bacterial appendages, **1:47–48**
bacterial movement, 1:52
biofilms, 1:67
sheathed bacteria, 2:514

Bacterial artificial chromosome (BAC), **1:48–49,** 1:242, 2:659
Bacterial epidemics. *See* Epidemics and pandemics; Epidemics, bacterial
Bacterial fossilization. *See* Fossilization of bacteria
Bacterial gastroenteritis, 1:236
Bacterial genetics. *See* Microbial genetics
Bacterial growth and division, **1:49–51,** 2:*473*
  agar and agarose, 1:5–7
  asexual generation and reproduction, 1:35–36
  carbon cycle, 1:101
  Coulter counter, 1:137–138
  culture, 1:144–145
  dilution theory and techniques, 1:156
  germ theory of disease, 1:28, 1:246–247, 1:273
  growth and growth media, 1:254–255
  metabolism, 2:376–377
  metal deposition in, 1:228
  Petri, Richard Julius, 2:430–431
  phospholipids, 2:435
  qualitative and quantitative techniques, 2:474–475
  respiration, 2:484–485
  sporulation, 2:527
  sterilization, 2:531–532
  synchronous growth, 2:537
  See also Colony and colony formation
Bacterial infection
  acne, 1:1–2
  anaerobic infection, 1:16–17
  animal models of infection, 1:*18,* 1:18–19, **1:18–19**
  antibiotic resistance, 1:25
  antibiotics, 1:24–25
  blood borne infections, 1:80–82
  botulism, 1:84–85
  brucellosis, 1:88–89
  bubonic plague, 1:93–95, 1:*94,* 1:188, 1:193–194, 1:195, 1:274
  campylobacteriosis, 1:99–100
  Centers for Disease Control (CDC), 1:110–112
  cholera, 1:193, 1:196
  chronic disease, 1:123–124
  diphtheria, 1:157–158
  dysentery, 1:168–170
  enterobacterial infections, 1:188–189
  enterotoxin and exotoxin, 1:189
  epidemics, 1:193–196
  epidemiology, 1:198–200
  Escherichia coli infection, 1:171, 1:201–202
  eye infections, 1:212–213
  gastroenteritis, 1:236
  germ theory of disease, 1:28, 1:246–247, 1:273
  gonorrhea, 1:251
  helicobacteriosis, 1:262
  immune system, 1:287–288
  infection and resistance, 1:308–310
  infection control, 1:310–311
  Koch's postulates, 1:247, 1:284, 1:327, 1:328, 1:353
  leprosy, 1:196, 1:346–348
  meningitis, 2:374–375
  mycobacterial infections, atypical, 2:406–407
  mycoplasma infections, 2:408
  nosocomial infection, 2:411–412
  pertussis, 2:429–430
  pneumonia, 2:444–445
  Q fever, 2:471–472
  Rickettsia and rickettsial pox, 2:492–493
  Salmonella food poisoning, 2:504–505, 2:558
  sexually transmitted diseases (STDs), 2:510–514
  skin infections, 2:516–517
  strep throat, 2:532–533
  sulfa drugs, 2:535
  T cells (T lymphocytes), 2:539–540
  tetanus, 2:543
  toxic shock syndrome, 2:529, 2:534, 2:547–548
  transmission of pathogens, 2:553
  tuberculosis, 1:111, 1:123, 1:168, 1:196, 2:555–557
  tularemia, 2:557–558
  typhoid fever, 2:558–560
  typhus, 2:560–561
  vaccine, 2:568–572
  zoonoses, 2:615–616
Bacterial kingdoms, **1:51**
  Archaea, 1:34
  autotrophic organisms, 1:39
  cladistics, 2:438
  marine microbiology, 2:365–366
  microbial symbiosis, 2:382–383
  microbial taxonomy, 2:383–384
  phylogeny, 2:437–438
  Prokaryotae, 2:450–451
  soil formation, involvement of microorganisms, 2:523
  See also Microbial taxonomy
Bacterial membrane transport. *See* Prokaryotic membrane transport
Bacterial membranes and cell wall, **1:51–52**
  electron transport system, 1:182
  lipopolysaccharide and its constituents, 1:351–352
  membrane fluidity, 2:373
  mycoplasma infections, 2:408
  periplasm, 1:51, 2:429, 2:453
  phospholipids, 2:435
  porins, 2:447–448
  prokaryotic membrane transport, 2:451–452
  protein export, 2:453–454
  signal hypothesis, 2:515–516
Bacterial meningitis, 1:195–196
Bacterial movement, **1:52**
  attractants and repellents, 1:37
  bacterial appendages, 1:47–48, 1:52, 1:67
  gliding bacteria, 1:249
  magnetotactic bacteria, 2:360–361
  marine microbiology, 2:365–366
  protein export, 2:453–454
  spirochetes, 2:525–526
Bacterial surface layers, **1:53**
  glycocalyx, 1:250
  phospholipids, 2:435
  protein export, 2:453–454
Bacterial ultrastructure, 1:*53,* **1:53–54**
  bacterial membranes and cell wall, 1:52
  bacterial surface layers, 1:53
  Bayer, Manfred E., 1:57–58
  Beveridge, Terrance J., 1:63
  cell membranes and cell wall, 1:51–52
  cytoplasm, 1:145–147
  electron microscopy examination of microorganisms, 1:180–181
  electron transport system, 1:182
  peptidoglycan, 1:25, 1:51, 2:427, 2:428, 2:429
  periplasm, 1:51, 2:429, 2:453
  phospholipids, 2:435

protein export, 2:453–454
protoplasts and spheroplasts, 1:230, 2:462
transduction, 2:549
See also Microscope and microscopy
Bacteriochlorophyll. *See* Photosynthesis
Bacteriocidal and bacteriostatic, **1:54–55**
disinfection and disinfectants, 1:158–160
sterilization, 2:531–532
Bacteriology. *See* Bacteria; Bacterial infection
Bacteriophage and bacteriophage typing, **1:55–56,** 2:582
bacteriophage lambda, 1:340
Berg, Paul, 1:60–62
lysogeny, 1:356–357
lytic replication, 2:583
phage genetics, 2:433–434
phage therapy, 2:434
Phi X 174, 2:516
T phage, 2:477
transduction, 2:549
See also Phage genetics; Phage therapy
Bacteriophage lambda, 1:340, 2:433, 2:577
Bacteriorhodopsin, 2:437
Bacteroides fragilis, 1:16
Bacteroides succinogenes, 1:100
Baer, Karl Ernst von, 2:646
Bailey, W.T., Jr., 2:654
Baillou, Guillaume de, 2:402
Bain, Barbara, 2:656
Balbiani, Edouard G., 2:649
Baltimore, David, **1:56–57,** 2:657
Bamberger, Eugen von, 1:339
Barré-Sinoussi, Françoise, 2:400
Base pairing, 2:*490*
Basidiomycetes, **1:57,** 2:407
hyphae, 1:284
lichens, 1:348, 2:407, 2:411
Basophils, 1:17
Bassi, Agostino, 1:247
Bateson, William, 2:650, 2:651
Bayer, Manfred E., **1:57–58**
Bayou virus, 1:259
BCG vaccine, 2:432, 2:555–556
Bdellovibrio bacteriovorans, 1:53
Beadle, George Wells, 1:175, 2:382, 2:541, 2:653, 2:656
Beaver fever. *See* Giardia and giardiasis
Beckwith, Jonathan Roger, **1:58**
Beggiatoa, 2:535
Beguin, Jean, 2:644
Behring, Emil von, **1:58–59,** 1:178, 1:272, 1:287, 1:325
Beijerinck, Martinus Willem, **1:59–60,** 1:316, 1:325, 2:528, 2:650, 2:651
Bell, Graham, 2:480
Benacerraf, Baruj, 1:288
Benda, Carl, 2:651
Benign endemic typhus, 2:492
Benzer, Seymour, 1:86, 2:655
Berg, Paul, **1:60–62,** 2:480, 2:657, 2:658
Bergey, David Hendricks, **1:62,** 2:451
Berkeley, Reverend M.J., **1:62–63**
Berry, George P., 2:653
Berson, Solomon A., 2:608
Beta globulins, 1:250
Beta hemolysis, 1:80, 1:*80*
Beta radiation, 2:479
Beveridge, Terrance J., **1:63**
Bevis, Douglas, 2:654
Bichat, Marie-François-Xavier, 2:646
Bifidobacterium bifidum, 2:381
Binary fission, 1:108
Binnig, Gerd, 1:37
Bioaugmentation, 1:73
Biochemical analysis techniques, **1:63–65**
buffer, 1:95–96
electrophoresis, 1:182–183
enzymes, 1:192–194
fluorescence *in situ* hybridization (FISH), 1:221–222, 2:415
protein crystallography, 2:452
See also Biochemistry; Dyes; Immunological analysis techniques; Laboratory techniques in immunology; Laboratory techniques in microbiology
Biochemistry, **1:65–66**
Berg, Paul, 1:60–62
Donnan equilibrium, 1:165–166
electron transport system, 1:182
hydrophobic and hydrophilic, 1:281–282
Krebs cycle, 1:331–332
metabolism, 2:376–377
Miller-Urey experiment, 2:389–390, 2:403
oxidation-reduction reaction, 2:417
pH, 2:433
phospholipids, 2:435
proteins and enzymes, 2:455–457
respiration, 2:484–485
RNA, 2:488–492
See also Biochemical analysis techniques
Biochips, 1:77
Biodegradable substances, **1:66–67**
biogeochemical cycles, 1:68–69
petroleum microbiology, 2:431–432
wastewater treatment, 2:590
Biofilm formation and dynamic behavior, **1:67–68**
anti-adhesion, 1:23–24
chronic bacterial disease, 1:123–124
contamination, 1:275
dental plaque, 2:442
glycocalyx, 1:250
quorum sensing, 1:68, 2:474
vinegar production, 1:175
wastewater treatment, 2:590
Biogas, 2:379
Biogeochemical cycles, **1:68–69,** 1:*69*
extraterrestrial microbiology, 1:209–211
fossilization of bacteria, 1:228
marine microbiology, 2:365–366
methane oxidizing and producing bacteria, 2:378–379
Biohazard, disposal of infectious microorganisms, 1:160–161
Bioinformatics and computational biology, **1:69–70,** 2:458
DNA chips and microarrays, 1:164
The Biological and Toxin Weapons Convention, 1:71
Biological warfare, **1:70–71**
Biological Weapons Convention (BWC), 1:71–72, 1:78
See also Bioterrorism
Biological weapons
biological warfare, 1:70–71
Biological Weapons Convention (BWC), 1:71–72, 1:75–76, 1:78
bioterrorism, 1:75–79
Biological Weapons Convention (BWC), **1:71–72,** 1:75–76, 1:78

Bioluminescence, 1:*72,* **1:72–73**
  dinoflagellates, 1:73, 1:156–157
  luminescent bacteria, 1:354
Bioremediation, **1:73–74,** 1:*74*
  biodegradable substances, 1:67
  economic uses and benefits of microorganisms, 1:174–175
  enzymes, 1:192–194
  petroleum microbiology, 2:*431,* 2:432
  radiation-resistant bacteria, 2:478–479
  zooplankton, 2:616–617
Biosensor technology, 1:77
Biotechnology, **1:74–75**
  bacterial artificial chromosome (BAC), 1:48–49
  bacteriophages and bacteriophage typing, 1:55–56
  biological warfare, 1:70–71
  bioremediation, 1:73–74
  bioterrorism, 1:75–77
  chemical mutagenesis, 1:114–115
  chemotherapy, 1:116–117
  contamination, 1:134
  enzymes, 1:192–194
  fluorescence *in situ* hybridization (FISH), 1:221–222, 2:415
  food preservation, 1:222–225
  food safety, 1:225–226
  The Institute for Genomic Research (TIGR), 2:544
  mutagenesis, 2:406
  proteomics, 2:457–458
  protoplasts and spheroplasts, 2:462
  spectroscopy, 2:524–525
  transmission electron microscope (TEM), 1:179–180, 2:388
  viral vectors in gene therapy, 2:578–579
Bioterrorism, **1:75–79,** 1:*78,* 2:660
  anthrax, 1:19–22, 1:241, 2:467
  fume hood, 1:229–230
  genetic identification of microorganisms, 1:77
  protective measures, 1:77–79
  smallpox, 2:521–522
  tularemia, 2:557–558
  See also Biological warfare; Genetic identification of microorganisms
Bioterrorism, protective measures, **1:77–79,** 2:660
  vaccine, 2:568–572
  Vozrozhdeniye Island, 2:585
Bioterrorism Preparedness and Response Program (BPRP), 1:79
Birdseye, Charles, 1:224
Birnaviruses, 2:580
Bischoff, Theodor Ludwig Wilhelm, 2:647
Bishai, William R., 2:660
Bishop, Michael J., 2:658
Black, Sir James, 1:185
Black bread mold, 1:232
Black Creek Canal virus, 1:259
Black Death. *See* Bubonic plague
Black flies, 2:423
"Black smokers," 1:282
Black tide, 2:470
Blackburn, Elizabeth, 2:658
Blackheads, 1:2
Blaese, Michael R., 2:659
Blakemore, Richard, 2:360
Blastomyces dermatitidis, 2:613
Blastomycosis, 2:613
Blattner, Frederick, 1:203
Blepharitis, 1:212
Blobel, Günter, 2:453, 2:515
Blood
  antigens, 1:28
  autologous banking, 1:38–39
  blood type, 1:28
  buffers in, 1:95–96
  contamination, 1:135
  See also Blood borne infections
Blood agar, hemolysis, and hemolytic reactions, 1:6, 1:*80,* **1:80,** 1:257
Blood borne infections, **1:80–82,** 1:*81*
  Chagas disease, 1:111–112
  meningitis, 2:374
Blood transfusions, antigens, 1:28
Blood type, antigens, 1:28
Bloom, Barry, 2:660
Blooms
  cyanobacteria, 1:82
  dinoflagellates, 1:157
  red tide, 1:156–157, 2:460, 2:481–482
  snow blooms, 2:522
Blue-green algae, **1:82–83,** 1:154, 1:203
  chlorophyll, 1:119
  chloroplast, 1:120
  fossils, 1:228
  gas vacuoles, 1:235
  lichens, 1:348, 2:407, 2:411
  photosynthesis, 2:436
Blue River virus, 1:259
Boiling, to sterilize, 2:531–532
Boinformatics and computational biology, 1:77
Bolivian hemorrhagic fever, 1:34, 1:263
Bordet, Jules, **1:83–84,** 1:287, 2:651
Bordetella pertussis, 2:429–430, 2:527
Borel, Jean-François, **1:84**
Borosilicate glass, 2:468
Borrelia burgdorferi, 1:82, 1:122, 1:123, 1:355–356, 2:526
Botox, 1:85
Botulism, 1:16, **1:84–85,** 2:576
Botulism toxin, 1:84–85
Boutonneuse fever, 2:493
Boveri, Theodor, 2:650, 2:651
Bovet, Daniele, 1:116
Bovine spongiform encephalopathy. *See* BSE and CJD disease
Boyer, Herbert Wayne, **1:85–86,** 2:481, 2:657
BPRP. *See* Bioterrorism Preparedness and Response Program
Bragg, Sir William Lawrence, 1:140
Brain-Hear Infusion (BHI) broth, 1:255
Bread mold, 1:232, 2:409
Brenner, Sydney, **1:86–87,** 1:141, 1:239, 2:375, 2:656
Brevitoxin, 1:157
Brewers yeast, 2:395
Bridges, Calvin Blackman, 2:652
Brill-Zinsser disease, 2:560
Brock, Thomas D., **1:88,** 2:540, 2:657
Broglie, Louis Victor de, **1:152–153,** 1:179–180, 2:388, 2:496
Bronchitis, 1:3
Brown algae, 1:6, 1:323–324, 2:421, 2:460
Brown-Sequard, Charles-Edouard, 2:650
Brown, D.R., 1:92
Brown, James, 2:652
Brown, Rachel, 1:260, 1:261
Brown, Robert, 2:646–647

Brownian motion. *See* Bacterial movement
Brucella, 1:206
Brucella abortus, 1:88, 1:89
Brucella melitensis, 1:88, 1:89
Brucella suis, 1:88, 1:89
Brucellosis, **1:88–89,** 1:206, 2:425
Bruton's agammaglobulinemia, 1:293
BSE, scrapie, and CJD, recent research advances, **1:92–93**
BSE and CJD disease, **1:89–93,** 1:*91,* 2:449
    ethical issues and socio-economic impact, 1:90–92
    food safety, 1:225–226
    prions, 2:449
    research advances, 1:92–93
BSE and CJD disease, ethical issues and socio-economic impact, **1:90–92**
Bubonic plague, **1:93–95,** 1:*94,* 1:188, 1:193–194, 1:274, 2:644
Buchanan, Robert Earle, **1:95,** 1:207
Buchner, Eduard, 1:66, 1:192, 1:217
Buchner, Hans, 1:66, 1:255, 1:287
Budding. *See* Yeast
Buescher, Edward Louis, 2:424
Buffer, **1:95–96,** 2:433
Bulb polio, 2:446
Bulbospinal polio, 2:446
Bull kelp, 1:323
Bunyavirus group, 1:263
Burke, David, 2:610
Burkholderia mallei, 1:70
Burkitt's lymphoma, 1:201, 2:558
Burnet, Frank Macfarlane, **1:96–97,** 1:175, 1:321, 2:370, 2:652, 2:654
BWC. *See* Biological Weapons Convention

C

C substance, 1:337
Caenorhabditis elegans, 1:87
CagA, 1:262
Caine, Roy, 1:184
Calanus, 2:616
Calmette, Leon Charles Albert, 2:432, 2:555
Calne, Donald, 1:288
Caloplaca, 1:349
Campylobacter jejuni, 1:99, 2:386
    food safety, 1:135, 1:226, 2:576
Campylobacteriosis, **1:99–100**
Cancer
    chemotherapy, 1:116–117
    as uncontrolled cell division, 1:105
Candida albicans, 1:100, 2:380, 2:609, 2:612, 2:*612*
Candida glabrata, 1:100, 2:612
Candida guilliermondi, 1:100
Candida krusei, 1:100
Candida parapsilosis, 1:100, 2:612
Candida tropicalis, 1:100
Candidiasis, **1:100,** 2:612
    skin infections, 2:516–517, 2:609
    thrush, 1:261, 2:546–547, 2:609
Canine distemper, 2:575
Canning, 1:224–225
Capsid, 1:3, 2:583
    See also Glycocalyx
Capsule, 1:48
Carageenan, 2:462
Carbon cycle in microorganisms, **1:100–101**
    aerobes, 1:5
    biogeochemical cycles, 1:68–69
    photosynthetic microorganisms, 2:437
    plankton and planktonic bacteria, 2:440–441
Careers in microbiology, **2:371–373**
Caries, 2:379–380
Carl Zeiss Foundation, 1:1
Caskey, Charles T., 2:657
Cassette mutagenesis, 2:406
Catecholamines, 1:16
Cauliflower mosaic virus (CMV), 2:515
Caullery, Maurice, 2:398
Caulobacter, **1:101**
Caulobacter crescentus, 1:67
Cb3 complement protein, 2:417
CD45, 2:428
CDC. *See* Centers for Disease Control
Cech, Thomas R., **1:101–103,** 2:659
Cell cycle and cell division, **1:103–105,** 1:*104,* 1:106, 1:243
    asexual generation and reproduction, 1:35–36
    eukaryotic, 1:106–108
    Golgi body, 1:251
    prokaryotic, 1:106–108
    See also Cell cycle (eukaryotic), genetic regulation of; Cell cycle (prokaryotic), genetic regulation of; Cell membrane transport
Cell cycle (eukaryotic), genetic regulation of, **1:106–108,** 1:*107,* 1:243
    chromosomes, 1:120–122
    nucleus, 2:412
    recombination, 2:481
    transgenics, 2:550–551
    See also Genetic regulation of eukaryotic cells
Cell cycle (prokaryotic), genetic regulation of, **1:108–109**
    chromosomes, 1:122–123
    mutations and mutagenesis, 2:405–406
    recombination, 2:481
    restriction enzymes, 2:485
    transgenics, 2:550–551
    translation, 2:551–553
    See also Genetic regulation of prokaryotic cells
Cell-mediated immune response. *See* Immunity, cell mediated
Cell membrane transport, **1:109–110,** 1:*110*
    electron transport system, 1:182
    Golgi body, 1:251
    hydrophobic and hydrophilic, 1:281–282
    lysosome, 1:357
    mitochondria and cellular energy, 2:392–393
    phospholipids, 2:435
    protein export, 2:453–454
Cell wall. *See* Bacterial membranes and cell wall
Cellular immunity, 2:362
Cellular slime molds, 2:518
Cellulitis, 2:516
Centers for Disease Control (CDC), 1:78–79, **1:110–112**
Central Dogma, 1:163, 2:*396*
Centrifugation, 1:64
Ceratium, 2:470
Cestodes, 2:423
CGD. *See* Chronic granulomatous disease
Chagas, Carlos, 1:111
Chagas disease, **1:111–112**
Chain, Ernst Boris, **1:112–113,** 1:116, 1:219, 1:276, 2:653
Chambers, Robert, 2:647

Chancroid, 2:512
Chang, Annie, 2:657
Chaperones, **1:113–114,** 1:261, 2:429, 2:582
Chargaff, Erwin, 2:595, 2:654
Charophyceae, 1:119
Chase, Martha Cowles, **1:114,** 1:269, 2:655
Chediak-Higashi syndrome (CHS), 1:297
Chemical mutagenesis, **1:114–115,** 1:161–164
See also Mutations and mutagenesis
Chemoautotrophic and chemolithotrophic bacteria, **1:115–116,** 2:451
carbon cycle, 1:101
extremophiles, 1:211–212
hydrothermal vents, 1:282–283
methane oxidizing and producing bacteria, 2:378–379
photosynthetic microorganisms, 2:437
sulfur cycle, 2:536
Winogradsky column, 2:601
Chemotaxis, 1:47
See also Bacterial movement
Chemotherapy, **1:116–117,** 2:416
Chermann, Jean-Claude, 2:400
Chiasmata, 1:105
Chickenpox, 2:572–573, 2:573
Childbed fever, 2:535
Chitin, **1:117–118,** 1:232
Chlamydia infection, 2:512
eye infections, 1:213
pneumonia, 1:118
Chlamydia pneumoniae, 1:118
Chlamydia psittaci, 1:118, 2:445
Chlamydia trachomatis, 1:118, 1:123
Chlamydial pneumonia, **1:118**
Chlamydomonas, 2:460
Chlamydomonas nivalis, 2:522
Chloramphenicol, typhoid, 2:560
Chlordexidine, as disinfectant, 1:159
Chlorella, 1:119
Chlorination, **1:119–120**
cysts, 1:119
wastewater treatment, 2:590
Chloroflexus auranticus, 1:249
Chlorophyceae, 1:119
Chlorophyll, **1:119**
Chlorophyta, 1:119–120
chloroplasts, 1:82, 1:120, 1:155
protozoans, 2:462
Chlorophyta, **1:119–120,** 2:460
lichens, 1:348, 2:407, 2:411
Chloroplasts, 1:82, **1:120,** 1:155, 2:436
Chlortetracycline, 1:116
Cholera, 1:193, 1:196, 1:327
Cholera toxin, 1:189
Chondrus, 2:488
Chorioretinitis, 1:212
Chromatium spp., 2:409
Chromatography, 1:64
Chromosome number defects, 1:121
Chromosomes, **1:120–123,** 2:550
See also Chromosomes, eukaryotic; Chromosomes, Prokaryotic
Chromosomes, eukaryotic, **1:120–122,** 2:387, 2:412
polymerase chain reaction (PCR), 2:446–447
recombinant DNA molecules, 2:480–481
Chromosomes, prokaryotic, **1:122–123**
bacteriophages and bacteriophage typing, 1:55–56
polymerase chain reaction (PCR), 2:446–447
recombinant DNA molecules, 2:480–481
yeast artificial chromosome (YAC), 2:610–611
Chronic bacterial disease, **1:123–124**
ear infections, 1:172
helicobacteriosis, 1:262
Chronic fatigue syndrome, mycoplasma, 2:408
Chronic gastritis, 1:262
Chronic granulomatous disease (CGD), 1:297
Chronic hepatitis, 1:264, 1:314
Chronic myelogenous leukemia (CML), 2:415, 2:416
Chrysochromulina polylepis, 2:482
CHS. *See* Chediak-Higashi syndrome
Chyrids, 1:232
Chytridiomycetes, 1:284, 2:407
cI protein, 1:356
Ciguatera, 1:157
Ciliates, 2:459
Ciliopa, 2:459
Ciprofloxacin, 2:467
Citric acid cycle, 2:484
CJD disease, **1:89–93**
ethical issues and socio-economic impact, 1:90–92
research advances, 1:92–93
See also BSE and CJD disease
Cladina alpestris, 1:349
Cladistics, 2:438
Cladocera, 2:616
Class A pipette, 2:439
Class B pipette, 2:439
Class I antigens, 2:554
Class I MHC genes, 2:361
Class I viruses, 2:577
Class II MHC genes, 2:361
Class II antigens, 2:554
Class II viruses, 2:577
Class III MHC genes, 2:361
Class III viruses, 2:577
Class IV viruses, 2:577
Class V viruses, 2:577
Class VI viruses, 2:577
Clinical microbiology. *See* Microbiology, clinical
Clonal deletion, 2:539
Clonal selection theory, 1:302
Cloning: applications to biological problems, **1:124,** 1:230, 2:412, 2:658, 2:660
embryo cloning, 1:75
molecular cloning, 1:75
plasmids, 1:200, 2:442–443
positional cloning, 1:75
shotgun cloning, 1:49, 1:242, 2:515
therapeutic cloning, 1:124
yeast artificial chromosome (YAC), 2:610–611
Cloroxybacteria, 2:436
Clostridium. *See* Botulism
Clostridium baratii, 1:84, 1:85
Clostridium botulinum, 1:16, 1:84–85, 2:576
Clostridium butyricum, 1:101
Clostridium difficile, 2:465
Clostridium perfringens, thermal death, 2:546
Clostridium tetani, 2:543
Club fungi, 1:232

CML. *See* Chronic myelogenous leukemia
CMV. *See* Cauliflower mosaic virus
Coagulase, **1:125**
Coccidia, 2:526
Coccidiosis, 2:464
Codons, 2:457, 2:491
Cofactors, defined, 1:72–73
Cohen, Stanley N., 1:86, **1:125–126,** 2:481, 2:657
Cohn, Ferdinand Julius, **1:127,** 1:326, 2:648, 2:649
Cold (common cold), **1:127–128,** 1:*128*
  enterovirus infections, 1:190
  home remedies, 1:129
  viruses, 1:128–129
Cold-loving bacteria, 2:466
Cold pasteurization, 2:427
Cold viruses, **1:128–129**
Coley, William, 1:286
Coliform bacteria. *See Escherichia coli*
Colitis, pseudomembranous, 2:465
Collins, Francis, 2:659
Colony and colony formation, **1:129–130**
  culture, 1:144–145
  dilution theory and techniques, 1:156
  germ theory of disease, 1:28, 1:246–247, 1:273
  growth and growth media, 1:254–255
  Pasteur, Louis, 2:424–426
  See also Bacterial growth and division
Colorado tick fever, 2:423
Colwell, Rita R., **1:130–131**
Combined immunodeficiency. *See* Immunodeficiency disease syndromes
Commensalism, 2:382
Commercial uses of microorganisms. *See* Economic uses and benefits of microorganisms
Common cold. *See* Cold
Common variable immunodeficiency, 1:294
Competent cells, 1:103
Competitive exclusion of bacterial adhesion. *See* Anti-adhesion methods
Complement, 1:26–27, **1:131,** 1:287, 2:416–417
Complement activation pathway, 1:131
Complement deficiency. *See* Immunodeficiency disease syndromes
Complement fixation tests, 1:83
Completed tests. *See* Laboratory techniques in microbiology
Complex media. *See* Growth and growth media
Composting, microbiological aspects, 1:*132,* **1:132–133**
  biogeochemical cycles, 1:68–69
  economic uses, 1:174–175
  soil formation, involvement of microorganisms, 2:523
Compound microscope. *See* Microscope and microscopy
Computational biology. *See* Bioinformatics and computational biology
Confirmed tests. *See* Laboratory techniques in microbiology
Confocal microscopy
  biofilms, 1:68
Congenital immunodeficiency, 1:293
Conjugation, 1:49, **1:133–134,** 2:459
Conjugation pili, 1:48
Conjunctivitis, 1:3, 1:212
Conseil, Ernest Alfred, 2:402
Conservative transposition, 2:554
Contact transmission, 1:308, 1:310
Contamination, 1:64, **1:134–136,** 1:*135,* 1:*136*
  by antiseptics, 1:31–32
  bacterial and viral, 1:135–136
  of bloodstream by *Candida,* 1:100
  Campylobacter jejuni, 1:99
  drinking water, 2:593
  E. coli O157:H7, 1:171
  Ebola virus, 1:173
  food safety, 1:225–226
  Giardia and giardiasis, 1:248–249
  hygiene, 1:283–284
  indicator species, 1:308
  luciferase reaction, 1:73
  mesophilic, 2:376
  mycobacteria, atypical, 2:407
  nosocomial infection, 2:411–412
  release prevention protocol, 1:134–135
  Salmonella food poisoning, 2:503–505, 2:558
  Shigella, 2:514–515
  transmission of pathogens, 2:553
  water pollution and purification, 2:591–592
  See also Contamination and release prevention protocol
Contamination and release prevention protocol, **1:134–135**
  fume hood, 1:229–230, 1:284
  Vozrozhdeniye Island, 2:585
Contamination, bacterial and viral, **1:135–136,** 1:*136*
Contractile vacuole, 2:531
Convention on the Prohibition of the Development, Production, and the Stockpiling of Bacteirological (Biological) and Toxin Weapons and on Their Destruction, 1:71
Cooking, food preservation, 1:224
Copeland, Herbert F., 2:458
Coronavirus, 1:129, 2:575
Correns, Carl, 2:651
Corticosteroids, allergies, 1:11
Cortisone, 1:218
Corynebacterium diphtheriae, 1:53, 1:136–137
Coryneform bacteria, **1:136–137**
Costerton, John William, **1:137**
Coulter counter, **1:137–138**
Coulter, Wallace H., 1:137
Cowie, Dean B., 1:343
Cowpox, 1:28, **1:138,** 1:272, 1:274, 1:287, 1:319, 2:584
Coxiella burnetii, 1:199, **2:471–472**
Coxsackie virus
  eye infections, 1:258
  hand-foot-mouth disease, 1:258
"Cradle cap," 2:517
Creighton, Harriet B., 2:653
Creutzfeldt-Jakob disease. *See* BSE and CJD disease
Crick, Francis, 1:87, 1:114, **1:138–141,** 1:*139,* 1:162, 1:239, 1:269, 2:655, 2:656
Crimean-Congo hemorrhagic fever, 1:263
cro protein, 2:433
"Cross-talk," 1:130
Croup, 1:10
Crustacea, 2:616
Crustose lichens, 1:348
Cryoprotection, **1:141–142**
  American Type Culture Collection (ATCC), 1:12–13
  desiccation, 1:154
Cryptococcal meningitis, 1:142, 1:143
Cryptococci and cryptococcosis, **1:142–143**
Cryptomonads, 2:469, 2:470, 2:488
Cryptosporidium and cryptosporidiosis, **1:143–144**
  cysts, 1:119
  drinking water, 2:594

Cryptosporidium muris, 1:143
Cryptosporidium parvum, 1:143, 1:236, 1:315, 2:526
Crystallography, proteins, 2:452, 2:*453*
CTLs. *See* Cytotoxic T-lymphocytes
Culture, 1:*144,* **1:144–145,** 1:335–336
defined, 1:49
dilution theory and techniques, 1:156
genetic identification of microorganisms, 1:240–241
planktonic bacteria, 2:441
Curing, 1:224
Cuvier, Georges, 2:646
Cyanobacteria, 1:82–83, 1:203, 2:436
chlorophyll, 1:119
chloroplast, 1:120
evolution of, 1:208
fossils, 1:228
gas vacuoles, 1:235
Cyclops, 2:616
Cyclosporin, 1:84, 1:307
Cystoseira osmundacea, 1:323
Cysts
chlorination, 1:119
Giardia, 1:249
protozoa, 2:423
Cytogenetics. *See* Molecular biology and molecular genetics
Cytokines, 1:104, 1:105, **1:145,** 1:291, 1:306, 1:313–314, 2:539
Cytokinesis, 1:107–108, 1:244
Cytomegalovirus, 1:33
Cytoplasm, eukaryotic, **1:145–146,** 1:*146*
Cytoplasm, prokaryotic, **1:146–147,** 2:551–553
Cytotoxic T-lymphocytes (CTLs), 2:539

D

D. immitis, 2:423
da Vinci, Leonardo, 2:644
Dalton, John, 2:646
Daraprim, 1:184
DARPA. *See* Defense Advanced Research Project
Darwin, Charles Robert, **1:150–151,** 2:647, 2:648
Darwin, Erasmus, 2:646
Dausset, Jean, 1:288
Davaine, C.J., 1:326
Davies, J.A.V., 2:538
Davies, Julian E., **1:151–152**
Davis, Bernard D., 2:654
Dawkins, Richard, 2:507
de Baillou, Guillaume, 2:402
de Broglie, Louis Victor, **1:152–153,** 1:179, 2:388, 2:496
de Jussieu, Antoine-Laurent, 2:646
de Maupertuis, Pierre-Moreau, 2:645
de Tournefort, Joseph Pitton, 2:645
de Vries, Hugo, 2:651
Dedrick, Helen M., 2:653
Defects of cellular immunity. *See* Immunodeficiency disease syndromes
Defense Advanced Research Project (DARPA), 1:77
Dehydration, 1:223
Deinococcus, 1:54, 2:532
Deinococcus radiodurans, 2:404
Deisenhofer, Johann, 2:436
Delayed immunity, 1:288–290
Delbrück, Max, 1:55, 1:268, 1:269, 1:321, 2:594, 2:653, 2:654, 2:657
Deletion mutations, 2:405, 2:406
Democritus, 2:643
Dendrogram, 2:383–384
Dengue fever, **1:153–154,** 1:263, 2:499
Denitrification, 2:411
Dental caries, **2:379–380**
Dental plaque, 1:17, 1:67, 2:387, 2:442
Deoxyribonucleic acid. *See* DNA
Dermatomes, 2:574
Dermatomycoses, 2:517
Descartes, René, 2:645
Desiccation, **1:154**
Desulfovibrio hydrocarbonoclasticus, 2:432
Detection of mutants. *See* Laboratory techniques in microbiology
d'Hérelle, Félix, 1:55, **1:149–150,** 1:356, 2:652
Diaptomus, 2:616
Diarrhea
Cryptosporidium parvum, 1:143, 1:236, 1:315
enterobacterial infection, 1:188
gastroenteritis, 1:236
giardiasis, 1:249
pseudomembranous colitis, 2:465
See also Dysentery; Gastroenteritis
Diarrhetic shellfish poisoning, 2:482
Diatomic nitrogen, 2:410
Diatoms, **1:154–155,** 2:421, 2:460, 2:*461,* 2:462, 2:482
Diauxy, 2:398
Dictyostelium, **1:155**
Dictyostelium discoideum, 1:130, 1:155, 2:518
Didanoside, 1:8
Diffusion, cell membrane transport, 1:109–110
DiGeorge syndrome, 1:294, 1:297
Dilution plating, 1:156
Dilution theory and techniques, **1:156**
Dinitrogen-fixing symbioses, 2:411
Dinoflagellates, **1:156–157,** 1:*181,* 2:460, 2:470
bioluminescence, 1:73
diarrhetic shellfish poisoning, 2:482
red tide, 1:156–157, 2:460, 2:481–482
Dinophysis, 2:481
Diphtheria, **1:157–158**
Behring, Emil von, 1:58–59, 1:178
coryneform bacteria, 1:136–137
history of, 1:59
Loeffler, Friedrich, 1:353
Schick, Bela, 2:505–506
Diphtheria toxin, 1:189
Directional selection, 2:506
Directly Observed Therapy (DOT), 2:557
Disease outbreaks. *See* Epidemics and pandemics
Disinfection and disinfectants, **1:158–160**
antiseptics, 1:31–32
chlorination, 1:118–119
contamination, 1:134
disposal of infectious microorganisms, 1:160–161
HACCP, 1:259–260
hygiene, 1:283–284
wastewater treatment, 2:590
Disposal of infectious microorganisms, 1:*160,* **1:160–161,** 2:590
Distemper, 2:575
DNA, **1:161–164,** 1:*162,* 1:*163,* 2:486, 2:550
acridine orange and, 1:2, 1:3
adenoviruses, 1:3
agarose, 1:7

antiviral drugs, 1:33
bacterial artificial chromosome (BAC), 1:48–49
base pairing, 2:*490*
Berg, Paul, 1:60–62
Brenner, Sydney, 1:86–87
Cech, Thomas R., 1:101–102
cell cycle and cell division, 1:103–105
chips and microarrays, 1:164
conjugation, 1:133
double helix, 1:162, 1:*162*
episomes, plasmids, insertion sequences, and transposons, 1:200
eukaryotes, 1:120–122, 1:204
genetic code, 1:238–240
hybridization, 1:165
The Institute for Genomic Research (TIGR), 2:544
life, origin of, 1:349–351
luciferase reaction, 1:73
lysogeny, 1:356–357
Miller-Urey experiment, 2:389–390, 2:403
mitochondrial DNA, 2:393
"naked" DNA, 1:10
nucleus, 2:412
origin of life, 1:351
phenotype and phenotypic variation, 2:435
plasmids, 1:200, 2:442–443
polymerase chain reaction (PCR), 2:446–447
protein crystallography, 2:452
proteins and enzymes, 2:455–457
radiation-resistant bacteria, 2:478–479
recombinant DNA, 1:60–62, 1:230, 2:480–481
recombination, 2:481
replication, 1:*163*
restriction enzymes, 2:485
retroposons and transposable elements, 2:485–486
shotgun cloning, 1:49, 1:242, 2:515
taq enzyme, 2:*540,* 2:540–541
transcription, 2:548–549
transduction, 2:439
transformation, 2:549–550
transposable elements, 2:485–486
transposition, 2:554
transposons, 2:554
Watson, James D., 1:114, 1:138, **2:594–595,** 2:*595*
See also Molecular biology and molecular genetics; Mutations and mutagenesis; Polymerase chain reaction
DNA chips and microarrays, **1:164,** 2:446–447, 2:458
DNA fingerprinting, 1:175, 2:658
DNA hybridization, **1:165**
cloning, 1:124
plasmids, 1:200, 2:442–443
polymerase chain reaction (PCR), 2:446–447
taq enzyme, 2:540–541
transgenics, 2:550–551
DNA phages, 1:55
DNA polymerase, 1:108, 2:540
Dobzhansky, Theodosius, 2:653
Dochez, Alphonse, 1:337
Dodge, Bernard O., 2:652
Doering, William E., 2:602
Dogs, viral infections of, 2:575
Domagk, Gerhard, 1:116, 2:535
Domoic acid, 2:482
Donnan equilibrium, **1:165–166**
Donnan, Frederick George, **1:165**
Doolittle, W. Ford, **1:166**
DOT. *See* Directly Observed Therapy
Double helix, 1:162, 1:*162*
Down syndrome, 1:121
Downy mildew, 2:461
Drinking water
water pollution and purification, 2:591–592
water quality, 2:592–594
Dropping pipette, 2:439
Dry heat sterilization, 2:532
Dry rot, 1:232
du Bois-Reymond, Emil, 1:73
Dubos, René, 1:116, **1:166–168**
Duclaux, Pierre, 2:649
Duggar, Benjamin Minge, 1:116
Dulbecco, Renato, 1:56, 2:655, 2:657
Dust mite, allergies, 1:11
Dutrochet, René-Joachim-Henri, 2:646
Dyes
acridine orange, 1:2–3, 1:24
fluorescent dyes, 1:222
See also Biochemical analysis; Laboratory techniques in immunology; Laboratory techniques in microbiology
Dysentery, **1:168–170,** 1:*169*
amebic dysentery, 1:11–12, 1:169, 1:186–187, 1:248–249, 2:423, 2:462
bacillary dysentery, 1:168, 2:514
Entamoeba histolytica, 1:169, 1:186–187
Shigella dysenteriae, 1:168, 2:514–515

E

E. coli. See Escherichia coli
Ear infections
chronic, **1:172**
Ebola hemorrhagic fever, 1:263
Ebola virus, 1:81, 1:*81,* **1:172–173,** 1:*173,* 1:264, 2:585, 2:657
Ecology of the oral cavity. *See* Microbial flora of the oral cavity
Ecology of the stomach and gastrointestinal tract. *See* Microbial flora of the stomach and gastrointestinal tract
Economic uses and benefits of microorganisms, **1:174–175**
biodegradable substances, 1:66–67
bioremediation, 1:73–74
chemical mutagenesis, 1:114–115
composting, 1:132–133
fermentation, 1:217–218
genetic engineering, 1:75
kelp, 1:323–324
lactic acid bacteria, 1:336
luminescent bacteria, 1:354
marine microbiology, 2:366
petroleum microbiology, 2:431–432
radioisotopes, 2:479–480
Rhodophyta, 2:488
wastewater treatment, 2:590
wine making, 2:599–601
See also Biotechnology
EcoRI, 2:485
Edelman, Gerald M., 1:29, **1:175–176,** 2:391
EGF. *See* Epidermal growth factor
Egregia menziesii, 1:323

Ehrlich, Paul, 1:59, **1:177–179,** 1:251, 1:253, 1:272, 1:274, 1:287, 1:289, 1:303, 2:511, 2:538, 2:650, 2:652
Eimeria, 2:464
Eklund, Carl, 2:464
Electromagnetic radiation, bacteriocidal methods, 1:54
Electron microscope
  examination of microorganisms, 1:180–181, 1:*181*
  Ruska, Ernst, 2:496
  scanning electron microscopy (SEM), 1:180, 2:388
  transmission electron microscope (TEM), 1:*179,* 1:179–181, 2:388
Electron microscope, transmission and scanning, **1:179–180**
Electron microscopic examination of microorganisms, **1:180–181,** 1:222
Electron paramagnetic resonance, 2:524
Electron spectroscopy for chemical analysis (ESCA), 2:525
Electron transfer chain, 2:484
Electron transport system, **1:182,** 2:377, 2:437
Electrophoresis, **1:182–183,** 1:333
  gel electrophoresis, 1:242
  immunoelectrophoresis, 1:298
  pulsed field gel electrophoresis, 1:122
  spectroscopy, 2:524–525
  See also Immunoelectrophoresis
Elephantiasis, 2:423
Elford, William J., 2:653
Elion, Gertrude Belle, 1:116, **1:183–185**
ELISA. *See* Enzyme-linked immunosorbant assay
Elk horn kelp, 1:323
Ellis, Emory L., 2:653
Embden, G., 1:217
Embryo cloning, 1:75
Empedocles, 2:643
Endemic typhus, 2:493, 2:560
Enders, John F., **1:185–186,** 2:369, 2:403, 2:500, 2:596, 2:654
Endocytosis, 1:109
Endoflagella, 1:48
Endometriosis, 2:483
Endotoxin, 1:351
Entamoeba dispar, 1:186, 1:315
Entamoeba histolytica, **1:186–187,** 1:315
  amebic dysentery, 1:11, 1:169, 2:423
  forms of, 1:12
Enterobacteriaceae, **1:187–188,** 2:386
  infections, 1:188–189, 1:236
  Shigella, 2:514–515
Enterobacterial infections, **1:188–189,** 1:236
Enteropathogenic *E. coli* (EPEC), 1:315
Enterotoxin and exotoxin, **1:189**
  blue-green algae, 1:82
  botulism, 1:84–85
  Clostridium difficile, 2:465
  E. coli infections, 1:171
  Enterobacteriaceae, 1:187
  food safety, 1:225–226
  lipopolysaccharide and its constituents, 1:351–352
  protein export, 2:453–454
  Pseudomonas, 2:466
  red tide, 2:481
  Salmonella, 2:505
  Shigella, 2:514–515
  toxic shock syndrome, 2:547–548
Enterotoxin B, 1:189
Enterovirus infections, **1:190,** 1:258
Enzyme induction and repression, **1:191–192**
Enzyme-linked immunosorbant assay (ELISA), 1:8, **1:190–191,** 1:*191,* 1:333–334
Enzyme repression, 1:192
Enzymes, **1:192–194,** 2:456
  acne and, 1:2
  Azotobacter, 1:41
  cell cycle, 1:243
  cell cycle regulation, 1:106
  cytokines, 1:145
  defined, 1:15
  glucose degradation, 2:484
  growth factors, 1:104, 1:106
  induction and repression, 1:191–192
  lysosome, 1:357
  phenol oxidase, 1:142–143
  restriction enzymes, 1:56, 1:61, 1:182, 1:183, 2:485
  structure of, 1:192
  taq enzyme, 2:540–541
  See also Proteins and enzymes
Enzymes, induction and repression, **1:191–192**
EPEC. *See* Enteropathogenic *E. coli*
Ephrussi, Boris, 2:397
Epidemic Information Exchange (Epi-X), 1:79
Epidemic parotitis. *See* Mumps
Epidemic typhus, 2:492, 2:493, 2:560
Epidemics. *See* Epidemics and pandemics; Epidemics, bacterial; Epidemics, viral; Epidemiology
Epidemics, bacterial, **1:195–196**
  bubonic plague, 1:93–95, 1:*94,* 1:188, 1:193–194, 1:195, 1:274
  cholera, 1:193, 1:196
  dysentery, 1:168–169
  epidemiology, 1:198–200
  leprosy, 1:196, 1:346–348
  meningitis, 1:195–196
  pertussis, 2:429–430
  syphilis, 1:251
  tuberculosis, 1:196
  See also Bacteria; Bacterial infection; Epidemics and pandemics; History of public health
Epidemics, viral, **1:196–198**
  AIDS, 1:7–9
  epidemiology, 1:198–200
  foot-and-mouth disease, 1:227–228, 1:354
  Great Flu Epidemic of 1918, 1:220–221
  hantavirus and Hanta disease, 1:198, 1:258–259
  hemorrhagic fevers, 1:197
  hepatitis, 1:264
  HIV, 1:197
  influenza, 1:193–194, 1:196–197, 1:220–221, 1:312
  smallpox, 1:196, 2:520–522
  yellow fever, 2:613–614
  See also Epidemics and pandemics; History of public health; Viral infection; Viruses
Epidemics and pandemics, **1:193–194,** 1:*194,* 1:*195,* 1:*197,* 1:*275*
  epidemiology, 1:198–200
  germ theory of disease, 1:28, 1:246–247, 1:273
  historical chronology, 2:643–660
  rinderpest, 2:648
  smallpox, 2:520, 2:521
  tracking diseases with technology, 1:199–200
  transmission of pathogens, 2:553
  See also Epidemics, bacterial; Epidemics, viral

Epidemiology, **1:198–200**
Centers for Disease Control (CDC), 1:110–112
epidemics, 1:196–198
foot-and-mouth disease, 1:227–228, 1:354
germ theory of disease, 1:28, 1:246–247, 1:273
hantavirus and Hanta disease, 1:258–259
Pasteur, Louis, 2:424–426
protozoa, 2:462–464
selection, 2:506–507
tracking diseases with technology, 1:199–200
tuberculosis, 2:555–557
See also Epidemics and pandemics; Infection control
Epidemiology, tracking diseases with technology, **1:199–200**
Epidermal growth factor (EGF), 1:104, 1:106
Epifluorescence microscopy, 1:222
Epiglottitis, 1:10
Episomes, plasmids, insertion sequences, and transposons, 1:200, 1:318
Epstein-Barr virus, **1:201,** 1:267, 2:558
blood borne infection, 1:82
mononucleosis, 1:201, 2:399
Erythema migrans (EM), 1:355
Erythroblastosis fetalis, 1:28, 1:340
Erythromycin, 1:118, 2:430
ESCA. *See* Electron spectroscopy for chemical analysis
Eschar-a sore, 2:493
Escherich, Theodor, 2:650
Escherichia coli, 1:174, 1:187, **1:201–202,** 1:*202,* 1:*254,* 2:386
anti-adhesion methods, 1:22, 1:23
bacterial artificial chromosome (BAC), 1:49
chronic disease, 1:123
colony, 1:129
detection of, 1:308
drinking water, 2:593
enteropathogenic *E. coli* (EPEC), 1:315
F genes, 1:133
foodborne illness, 1:225, 1:226
genetic studies, 1:123, 2:660
growth of, 1:3, 1:49, 1:254–255
0157:H7 infection, 1:171, 1:203, 1:226, 2:658
Hfr strains, 1:133
matrix porin, 1:*110*
phages, 1:55
porins, 2:447, 2:*448*
protein export, 2:454
sterilization, 2:531
thermal death, 2:546
transmission, 2:576
water pollution and purification, 2:591–592
Escherichia coli 0157:H7 infection, **1:171,** 1:203, 2:658
Ethyl alcohol, 1:218
Eubacteria, 1:51, **1:203,** 2:450
Eubacteriobonta, 2:450
Euglenoids, 2:460
Euglenophyta, 2:460
Eukaryotes, 1:*204,* **1:204–205,** 2:387
cell cycle and cell division, 1:103–105
cellular respiration, 2:484
chromosomes, 1:120–122, 2:387, 2:412, 2:446–447, 2:480–481
DNA, 1:161
genetic regulation, 1:106–108, 1:242–244
glycocalyx, 1:47–48, 1:68, 1:228, 1:250
paramecia, 2:421–422
protein synthesis, 2:454–455
Protista, 2:458–462
retroposons, 2:486
See also Cell cycle (eukaryotic), genetic regulation of; Chromosomes, eukaryotic; Cytoplasm, eukaryotic; Eukaryotic chromosomes; Genetic regulation of eukaryotic cells; Mycology
Eukaryotic chromosomes. *See* Chromosomes, eukaryotic
Euler-Chelpin, Hans von, **1:205–206**
Evans, Alice, **1:206–207,** 2:425
Evolution and evolutionary mechanisms, **1:207–208**
Archaea, 1:34
biogeochemical cycles, 1:68–69
cladistics, 2:438
conjugation, 1:133–134
Darwin, Charles, 1:150–151
DNA hybridization, 1:165
evolutionary origin of bacteria and viruses, 1:208–209
gene, 1:237–238
Miller-Urey experiment, 2:389–390, 2:403
Murchison meteorite, 2:403
phylogeny, 2:437–438
radiation mutagenesis, 2:477–478
rare genotype advantage, 2:480
selection, 2:506–507
See also Life, origin of
Evolutionary origin of bacteria and viruses, **1:208–209**
Archaea, 1:34
biogeochemical cycles, 1:68–69
DNA hybridization, 1:165, 1:170
life, origin of, 1:349–351
Miller-Urey experiment, 2:389–390, 2:403
Murchison meteorite, 2:403
mutants: enhanced tolerance or sensitivity to temperature and pH ranges, 2:404–405
photosynthesis, 2:436–437
photosynthetic microorganisms, 2:437
phylogeny, 2:437–438
Prokaryotae, 2:450–451
radiation mutagenesis, 2:477–478
rare genotype advantage, 2:480
selection, 2:506–507
See also Fossilization of bacteria; Life, origin of
Exobiology, 1:343
Exotoxin. *See* Enterotoxin and exotoxin
Extraterrestrial microbiology, **1:209–211,** 1:*210*
extremophiles, 1:211–212
fossils, 1:228
Murchison meteorite, 2:403
Extremophiles, **1:211–212,** 1:*212*
acid-loving bacteria, 1:211
alkaline-loving bacteria, 1:211
autotrophic organisms, 1:39
Brock, Thomas D., 1:88
chemoautotrophic and chemolithotrophic bacteria, 1:115–116
evolution of, 1:208
extraterrestrial microbiology, 1:209–211
halophilic bacteria, 1:211
hydrothermal vents, 1:282–283
Jannasch, Holger Windekilde, 1:319
marine bacteria, 2:366
mesophilic bacteria, 2:376
methane oxidizing and producing bacteria, 2:378–379
pH, 2:433

psychrophilic bacteria, 2:466
radiation-resistant bacteria, 2:478–479
sensitivity to pH and temperature, 2:404–405
sulfur cycle, 2:536
taq enzyme, 2:540–541
thermophiles, 1:211
See also Mutants: enhanced tolerance or sensitivity to temperature and pH ranges
Eye infections, 1:33, **1:212–213**

F

F genes, 1:133
F-pili, 1:48
Fabaceae, 2:411
Fabrizzi, Girolamo, 2:644
Facilitated diffusion, 1:109
See also Cell membrane transport
Fairy shrimp, 2:616
Famciclovir, 1:33
Fannie, Angelina, 2:649
Fansidar, 1:184
Farber, Sidney, 1:116
Fatal familial insomnia (FFI), 1:90, 2:520
Fauci, Anthony S., **1:215–216**
Feather boa kelp, 1:323
Feldman, Harry Alfred, **1:216–217,** 1:252
Feline leukemia, 2:487
Feline leukemia virus (FELV), 2:487, 2:575
Fermentation, 1:174, 1:*217,* **1:217–218,** 2:377
carbon cycle, 1:100–101
defined, 1:224
mold, 2:394–395
wine making, 2:599–601
Fertility. *See* Reproductive immunology
Fertility genes (F genes), 1:133
FFI. *See* Fatal familial insomnia
FGF. *See* Fibroblastic growth factor
Fibrinolysin, 1:125
Fibroblastic growth factor (FGF), 1:104, 1:106
Field ion microscope, 1:180
Filiarisis, 2:423
Filoviruses, 1:172–173, 1:263
Filtration, 1:54, 2:532
FimH, 1:23
Finger, Ernest, 1:339
Finlay, Carlos, 2:545
Firefly, bioluminescence, 1:73
Fischer, Emil, 1:192, 1:339
FISH. *See* Fluorescence *in situ* hybridization
Flagella, 1:48, 1:52
Flagellates, 2:459
Flagellin, 1:48
Flash pasteurization, 2:426–427
Flaviviridae, 2:536
Flaviviruses, 1:263, 2:585
Fleas, 2:423
Fleming, Alexander, 1:112, 1:116, **1:218–219,** 1:*219,* 1:274, 1:276, 2:427, 2:*428,* 2:604, 2:648, 2:653
Flemming, Walther, 2:649, 2:650
Flesh-eating disease, 2:534
Flexner, Simon, 2:494
Florey, Howard Walter, 1:112, 1:116, **1:219–220,** 1:276, 2:427, 2:653
Flu
epidemics and pandemics, 1:193–194, 1:312
Great Flu Epidemic of 1918, 1:220–221
hemagglutinin(HA) and neuraminidase (NA), 1:262–263
See also Influenza
Flukes, 2:423
Fluorescein isothiocyanate, 1:286
Fluorescence *in situ* hybridization (FISH), **1:221–222,** 2:415
Fluorescence microscopy, acridine orange and, 1:2–3
Fluorescent dyes, 1:65, **1:222**
acridine orange, 1:2–3, 1:24
antibiotic resistance tests, 1:23–24
See also Biochemical analysis; Laboratory techniques in immunology; Laboratory techniques in microbiology
Fluorescent probes, 2:415
Folic acid, 1:116
Foliose lichens, 1:348–349
Folliculitis, 2:516
Food additives, 1:225
Food preservation, **1:222–225**
Appert, Nicolas François, 1:33–34
enzymes, 1:192–194
mold, 2:394–395
pasteurization, 1:54, 1:246, 1:272, 2:426–427, 2:532, 2:569
Salmonella, 2:503–505
yeast, infectious, 2:612–613
Food safety, **1:225–226,** 1:*226*
Appert, Nicolas François, 1:33–34
Campylobacter jejuni, 1:99–100
dinoflagellates, 1:157
E. coli O157:H7 infection, 1:171
enzymes, 1:192–194
food preservation, 1:222–225
HACCP, 1:259–260
mold, 2:394–395
pasteurization, 1:54, 1:246, 1:272, 2:426–427, 2:532, 2:569
Salmonella, 2:503–504
Shigella, 2:514–515
toxoplasmosis, 2:548
yeast, 2:612–613
Foodborne illnesses, 1:225, 1:226
paralytic shellfish poisoning, 1:157, 2:482
Salmonella, 2:503–504, 2:558
staphylococci, 2:530
Foot-and-mouth disease, 1:*227,* **1:227–228,** 1:354
Forensic identification of microorganisms. *See* Genetic identification of microorganisms
Formaldehyde, as disinfectant, 1:159
Fossilization of bacteria, **1:228**
diatoms, 1:154–155
photosynthetic microorganisms, 2:437
See also Evolutionary origin of bacteria and viruses
Fox, George E., 2:658
Fracastoro, Gerolamo, 1:273, 2:644
Fraenkel-Conrat, Heinz, 2:655
Frameshift mutations, 2:405
Francisella tularensis, 2:557
Franek, Frantisek, 1:176
Franklin, Rosalind Elsie, 1:162, 2:595, 2:598, 2:655
Freeze-drying, 1:223
Freezing, 1:224
Frerichs, Friedrich von, 1:177
Freund, Jules, 2:653

Freund's Complete Adjuvant, 1:5
Friedewald, W.F., 2:495
Friend, Charlotte, 1:228–229, 1:*229*
Friend virus, 1:229
Frosch, Paul, 1:227, 1:354, 2:651
Fructose lichens, 1:349
Frustules, 1:155
Fucalean alga, 1:323
Fucoxanthin, 2:460
Fuligo septica, 2:519
Fume hood, **1:229–230,** 1:284
Funch, Peter, 2:659
Fungal genetics, **1:230–231**
  See also Microbial genetics
Fungal infection
  candidiasis, 1:100, 2:516–517, 2:609, 2:612
  eye infections, 1:213
  fungicides, 1:232
  infection control, 1:310–311
  skin, 2:517
  thrush, 1:261, 2:546–547
Fungi, 1:*231,* **1:231–232,** 2:387
  aerobes, 1:5
  Armillaria ostoyae, 1:35
  basidomycetes, 1:57
  Berkeley, Rev. M.J., 1:62–63
  candidiasis, 1:100
  chitin, 1:117–118
  colony and colony formation, 1:129–130
  cryoprotection, 1:141–142
  cryptococci and cryptococcosis, 1:142–143
  hyphae, 1:284
  lichens, 1:348–349, 2:407, 2:411
  mesophilic, 2:376
  mycelia, 1:230, 1:231, 2:394, 2:406
  Saccharomyces cerevisiae, 2:501
  sensitivity to temperature and pH ranges, 2:404
  Sick Building Syndrome, 2:408
  See also Fungal genetics; Fungal infection; Fungicides; Mold; Mycology
Fungicides, 1:158–160, **1:232**
Funk, Casimir, 2:652
Furious rabies, 2:476–477
Furth, Jacob, 1:229

G

Galen, 2:644
Gallagher, Robert E., 1:234
Gallo, Robert C., 1:7, **1:233–235,** 2:401, 2:487, 2:658
Gambierdiscus toxicus, 1:*181*
Gamma globulins, 1:250
Gamma hemolysis, 1:80
Gamma radiation, 2:479
Ganciclovir, 1:33
Ganders, 2:465
Garrod, Archibald Edward, 1:192, 2:651
Gas gangrene, 1:16
Gas vacuoles and gas vesicles, **1:235**
Gasohol, 1:218
Gastritis, chronic, 1:262
Gastroenteritis, 1:236
  adenoviruses, 1:3
  campylobacteriosis, 1:99–100
  Enterobacteriaceae, 1:187–188
  rotavirus, 1:236
  Sporozoa, 2:526
  See also Microbial flora of the stomach and gastrointestinal tract
Gastrointestinal tract. *See* Microbial flora of the stomach and gastrointestinal tract
GBS. *See* Guillain-Barre syndrome
Gel electrophoresis, 1:122, 1:242
Gelactose, 1:6
Gelidiuim, 2:488
Gelidium comeum, 1:6
Gene, **1:236–238,** 2:489
  Berg, Paul, 1:60–62
  oncogene, 1:104, 1:243, 2:415, 2:558
  restriction enzymes, 2:485
  See also Genetic identification of microorganisms; Genotype and phenotype; Mutations and mutagenesis
Gene amplification
  restriction enzymes, 2:485
  yeast artificial chromosome (YAC), 2:610–611
Gene chips. *See* DNA chips and microarrays
Gene flow, 1:207
Gene splicing, 1:85
Gene therapy, 1:60–62, 2:510, 2:578–579
Genetic code, 1:162, **1:238–240,** 1:*239,* 2:550
  Berg, Paul, 1:60–62
  gene, 1:237–238
  genotype and phenotype, 1:245–246
  operon, 1:237, 1:354, 2:398, 2:416
  proteins, 1:15
  restriction enzymes, 2:485
Genetic diseases, 1:121
Genetic diversity, conjugation, 1:133
Genetic drift, 1:207–208
Genetic engineering, 1:75, 1:134
Genetic identification of microorganisms, **1:240–241**
  bioterrorism, 1:77
  gene, 1:237–238
  genetic code, 1:239–240
  genotype and phenotype, 1:245–246
  microbial taxonomy, 2:383–384
  restriction enzymes, 2:485
  See also DNA; Microbial genetics
Genetic mapping, 1:*241,* **1:241–242**
  gene, 1:237–238
  The Institute for Genomic Research (TIGR), 2:544
  restriction enzymes, 2:485, 2:*485*
Genetic regulation of eukaryotic cells, **1:106–108, 1:242–244**
  cell cycle, 1:106–108
  DNA, 1:161–164
  recombinant DNA molecules, 2:480–481
  restriction enzymes, 2:485
  RNA, 2:488–492
  See also Cell cycle (eukaryotic), genetic regulation of
Genetic regulation of prokaryotic cells, **1:244–245**
  bacteriophages and bacteriophage typing, 1:55–56
  cell cycle, 1:108–109
  DNA, 1:161–164
  recombinant DNA molecules, 2:480–481
  transcription, 2:548–549
  translation, 2:551–553
  See also Cell cycle (prokaryotic), genetic regulation of

Genetic screening, karyotype analysis, 1:121
Genetically engineered vaccines. *See* Vaccine
Genetics
defined, 1:161
history of, 1:161–163
See also DNA; Fungal genetics; Gene; Genetic code; Genetic identification of microorganisms; Genetic mapping; Genetic regulation; Genotype and phenotype; Immunogenetics; Microbial genetics; Molecular biology and molecular genetics; Mutations and mutagenesis; Phage genetics; Phenotype and phenotypic variation; Proteomics; Radiation mutagenesis; Recombination; RNA; Transgenics; Transplantation genetics; Viral genetics
Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare, 1:71
Gengou, Octave, 1:83–84, 2:651
Genital herpes, 2:511–512, 2:513
Genital warts, 2:513
Genome Database, 2:659
Genomics, bioinformatics and computational biology, 1:69–70
Genotype and phenotype, **1:245–246**
gene, 1:237–238
phenotypic variation, 2:435
transduction, 2:439
See also Phenotype and phenotypic variation
Gentian violet, 1:253
Gépnéthon, French, 2:659
Gerber, Christoph, 1:36
Gerhardt, Karl, 1:177
Germ theory of disease, 1:28, **1:246–247,** 1:*247,* 1:273
immune system, 1:287–288
Koch's postulates, 1:247, 1:274, 1:327, 1:328, 1:353
Pasteur, Louis, 2:424–426
German measles, 2:597
Gerstmann-Sträussler-Scheinder (GSS) syndrome, 1:90, 2:520
Giant kelp, 1:323, 2:462
Giardia and giardiasis, 1:*248,* **1:248–249,** 2:367, 2:423, 2:462
Giardia lamblia, 1:249, 2:367, 2:531
Gibbs, J. Willard, 1:165
Gierer, Alfred, 2:655
Gilbert, Walter, 2:657, 2:658
Glanders disease, 1:70, 1:353
Gleevec, 2:416
Gliding bacteria, 1:52, **1:249**
Globulins, **1:249–250**
Glutaraldehyde, as disinfectant, 1:159
Glycocalyx, 1:47–48, 1:68, 1:228, **1:250**
Glycolysis, 2:484
Gold, Lois Swirsky, 1:13
Goldin, Barry, 1:337
Golgi, Camillo, **1:250–251,** 2:648
Golgi body, **1:251**
Gonococcus, 1:251
Gonorrhea, **1:251,** 2:510, 2:512
Gonyaulax, 2:470
Goodpasture, Ernest William, **1:252,** 2:402
Goodpasture's syndrome, 1:252
Gorbach, Sherwood, 1:337
Gorer, Peter, 2:554
Goss, Raymond, 2:595
Gotschlich, Emil Carl Anton Constantin, 1:252
Gotschlich, Emil Claus, **1:252**
Gowans, C.S., 1:288
Grabar, Petr Nikolaevich, 1:298
Gracilaria verrucosa, 1:6
Graffky, Georg, 1:353
Gram, Hans Christian Joachim, 1:53, **1:253,** 2:650
Gram-negative bacteria, 1:51, 1:53, 1:189, 1:253, 2:373, 2:447
Gram-positive bacteria, 1:51, 1:53, 1:158, 1:253, 2:373, 2:412, 2:428, 2:462
Gram staining, **1:253–254,** 1:335
Grana, 1:120
Great Flu Epidemic of 1918, 1:193, 1:220–221
Green algae, 2:460
Gregaria, 2:526
Greider, Carol, 2:658
Gresser, Ion, 1:288
Griffith, Frederick, 1:338, 2:382, 2:652
Griffith, J.S., 2:464
Group A streptococci, 2:534
Group B streptococci, 2:534–535
Group G streptococci, 2:535
Growth and growth media, 1:*254,* **1:254–255,** 1:335–336
agar and agarose, 1:5–7
blood agar, 1:80
Petri, Richard Julius, 2:430–431
Growth factors, 1:104, 1:106
Gruber, Max von, **1:255–256,** 1:339
Gruber-Widal test, 1:255
GSS syndrome. *See* Gerstmann-Sträussler-Scheinker syndrome
Guanarito virus, 1:34
Guerin, Camille, 2:432, 2:555
Guignard, Jean-Louis-Léon, 2:650
Guillain-Barre syndrome (GBS), 1:313, 2:574
Gymnodium, 2:470

H

HAARD. *See* Highly Active Anti-Retroviral Therapy
HACCP. *See* Hazard Analysis and Critical Control Points Program
Haddow, Alexander, 1:116
Hadlow, William, 2:464
Haeckel, Ernst, 2:458, 2:648
Haemophilus, 1:10, **1:257**
Haemophilus aegyptius, 1:257
Haemophilus ducreyi, 1:257, 2:512
Haemophilus influenzae, 1:195, 1:257, 2:374–375
genome, 2:659
history, 2:432
protein export, 2:454
Hair follicle, acne and, 1:1, 1:2
Hairpin telomere, 1:123
Hairy cell leukemia virus, 2:493
Haldane, J.B.S., 1:350
Halimeda, 1:119
Haller, Albrecht von, 2:645
Halobacteria, 2:436–437
Halobacterium NRC-1, 1:235
Halobacterium salinarum, 1:211
Halococcus, 1:255
Halophilic bacteria, 1:211
Halorhodopsin, 2:437
Hamilton, Robert, 2:402
HAN. *See* Health Alert Network
Hancock, Robert Ernest William, **1:257–258,** 2:447

Hand-foot-mouth disease, **1:258**
Hand washing, 1:283, 1:*283*
Hansen, Christian, 2:609
Hansen's disease. *See* Leprosy
Hansma, Paul, 1:37
Hantavirus and Hanta disease, 1:198, **1:258–259,** 2:659
Hantavirus pulmonary syndrome (HPS), 1:258, 1:259, 1:263
Hantzsch, Arthur, 1:339
Hartwell, Lee, 2:611
Harvey, William, 1:246, 2:644
Hata, Sahachiro, 2:538
HAV. *See* Hepatitis and hepatitis viruses
Hay fever, 1:10, 1:*11*
Hayes, William, 1:133, 2:655
Hazard Analysis and Critical Control Points Program (HACCP), **1:259–260**
Hazen, Elizabeth, **1:260–261**
HBV. *See* Hepatitis and hepatitis viruses
HCV. *See* Hepatitis and hepatitis viruses
HDV. *See* Hepatitis and hepatitis viruses
Health Alert Network (HAN), 1:79
Hearst, John, 1:102
Heart disease, *Chlamydia trachomatis,* 1:123
Heartworm, 2:423
Heat, bacteriocidal methods, 1:54
Heat shock proteins, 1:113, 1:261
Heat shock response, 1:47, **1:261**
Heating, for food preservation, 1:224
Heatley, Norman, 2:427
Heavy mutagenesis, 2:406
Heber-Katz, Ellen, 2:660
Heidelberger, Charles, 1:116
Heidelberger, Michael, 1:287
Helicobacter pylori, 1:262, 2:366, 2:381, 2:386
Helicobacteriosis, **1:262**
Helminths, 2:423, 2:597
Helper T cells, 1:288
Hemagglutinin (HA) and neuraminidase (NA), **1:262–263**
Hemolysis and hemolytic reactions, 1:80
Hemophiliacs, blood borne infection, 1:82
Hemophilus. See Haemophilus
Hemorrhagic colitis, 1:171
Hemorrhagic fever with renal syndrome (HFRS), 1:258, 1:259
Hemorrhagic fevers and diseases, 1:80–82, **1:263–264**
  arenavirus, 1:34–35
  dengue fever, 1:153–154, 1:263, 2:499
  Ebola virus, 1:172–173
  epidemics, 1:197
  hantavirus and Hanta disease, 1:258–259
  as public health issue, 2:467
Henle, Friedrich Gustav, 2:647
HEPA filter. *See* Fume hood
Hepadnaviruses, 1:264, **1:264,** 2:584
Hepatitis and hepatitis viruses, **1:264–267,** 1:*265,* 2:513
  chronic, 1:264, 1:314, 2:558
  hepatitis A virus (HAV), 1:264, 2:580
  hepatitis B virus (HBV), 1:264, 1:265–266, 2:508, 2:558, 2:584
  hepatitis C virus (HCV), 1:264, 1:266–267, 2:508
  hepatitis D virus (HDV), 1:267
  hepatitis E virus (HEV), 1:264, 1:267
  hepatitis G virus (HGV), 1:267
  seroconversion, 2:508
  vaccine, 1:265, 2:513
Herpes and herpes virus, **1:267–268,** 1:340, 2:513, 2:581, 2:584
  blood borne infection, 1:82
  chemotherapeutic agent, 1:116
  Epstein-Barr virus, 1:82, 1:201, 1:267, 2:399, 2:584
  herpes zoster virus, 1:212
  HHV4, 1:267
  HHV5, 1:267
  HHV6, 1:267
  HHV7, 1:267
  HHV8, 1:267
  HSV-1 (HHV1), 1:267
  HSV-2 (HHV2), 1:267
Herpes zoster virus (HZV), 1:212
Herpetosiphon, 1:249
Hershey, Alfred Day, 1:114, **1:268–270,** 2:654, 2:655, 2:657
Hertwig, Wilhelm, 2:649, 2:650
Hesse, Walter, 2:649
Heterokaryon, 1:230
Heterotrophic bacteria, 1:203, 1:255, 1:*270,* **1:270**
HEV. *See* Hepatitis and hepatitis viruses
Hfr strains, *Escherichia coli,* 1:133
HFRS. *See* Hemorrhagic fever with renal syndrome
HGV. *See* Hepatitis and hepatitis viruses
HHV. *See* Herpes and herpes virus
High efficiency particulate air filter. *See* Fume hood
Highly Active Anti-Retroviral Therapy (HAART), 1:9
Hildegard of Bingen, 2:644
Hillier, James, 1:180
Hinshelwood, Cyril, 1:86
Hinton, William A., 2:538
Hippocrates, 2:643
Histamine, 1:11, 1:16, **1:270–271**
Histocompatibility, **1:271,** 1:280
History of development of antibiotics, 1:116, 1:*276,* **1:276–277**
  Fleming, Alexander, **1:218–219**
  germ theory of disease, 1:28, 1:246–247, 1:273
  nosocomial infections, 2:411–412
  penicillin, 2:427, 2:511
  sulfa drugs, 2:535
History of immunology, **1:271–273**
  Avery, Oswald Theodore, 1:39–41
  historical chronology, 2:643–660
  Koch's postulates, 1:247, 1:274, 1:327, 1:328, 1:353
  measles, 2:368–369
  mumps, 2:402–403
  Schick, Bela, 2:505–506
History of microbiology, **1:273–274,** 1:*274*
  DNA, 1:161–163
  fermentation, 1:217–218
  food preservation, 1:222–225
  food safety, 1:224–225
  germ theory of disease, 1:28, 1:246–247, 1:273
  historical chronology, 2:643–660
  Koch's postulates, 1:247, 1:274, 1:327, 1:328, 1:353
History of public health, **1:274–276,** 1:*275*
  Centers for Disease Control (CDC), 1:110–112
  chemotherapy, 1:116–117
  food preservation, 1:222–225
  germ theory of disease, 1:28, 1:246–247, 1:273
  gonorrhea, 1:251
  Koch's postulates, 1:217, 1:247, 1:274, 1:328, 1:353
  measles, 2:368–369
  mumps, 2:402–403

nosocomial infections, 2:411–412
pertussis, 2:429–430
poliomyelitis and polio, 2:445–446
Sabin, Albert, 2:499–501
Salk, Jonas, 2:501–503
sexually transmitted diseases, 2:510–512
sulfa drugs, 2:535
World Health Organization (WHO), 2:603–604
See also Epidemics, bacterial; Epidemics, viral; Epidemics and pandemics; Epidemiology
Hitchings, George Herbert, 1:116, 1:184, 1:185
HIV. *See* Human immunodeficiency virus
Hives, 1:*311*
HLA. *See* Human leukocyte antigen
Hobby, Gladys Lounsbury, **1:277**
Hoffmann, Erich, 2:538
Hog cholera. *See* Swine fever
Holley, Robert, 1:325
Holmes, Francis O., 2:652
Homologous chromosomes, 1:104–105
Homologues, 1:104–105
Homoserine lactone, 2:474
Honey mushroom, 1:35
Hong Kong flu, 1:196–197
Hood, Leroy, 2:465, 2:658
Hooke, Robert, 1:246, **1:277–278,** 2:388, 2:645
Hopkins, Frederick Gowland, 1:331
Hormones, 1:16
Horne, Robert W., 2:656
Horowitz, Norman H., 1:351
Hospitals, infection control, 1:310
Hot tub folliculitis, 2:516
HPS. *See* Hantavirus pulmonary syndrome
HPV. *See* Human papillomavirus
HSV. *See* Herpes and herpes virus
HTLV. *See* Human T-cell leukemia virus
Huang, Alice Shih-Hou, 1:56, **1:278–279**
Huber, Robert, 2:436
Huggins, Charles, 1:116
Hughes, Arthur, 1:140
Human Genome Organization (HUGO), 2:659
Human Genome Project, 1:164, 2:516, 2:574, 2:575, 2:659, 2:660
Human granulytic ehrlichiosis, 1:82
Human immunodeficiency virus (HIV), **1:279–280,** 1:*295,* 1:340–341, 2:487, 2:493, 2:512
antiviral drugs, 1:33
chemotherapy, 1:116
epidemics, 1:197
history of, 1:7, 2:401
mechanism of action, 2:519
seroconversion, 2:508
See also AIDS
Human leukocyte antigen (HLA), 1:27, **1:280,** 1:288, 2:363, 2:554
Human papillomavirus (HPV), 2:516, 2:558, 2:584
Human T-cell leukemia virus (HTLV), 1:233, 1:281, 2:493, 2:519, 2:558, 2:585
Humoral immune response. *See* Immunity, humoral regulation
Humoral immunity, 2:361, 2:539
Hungate, Robert, 2:655
Hunter, John, 1:319
Huxley, Thomas Henry, 2:648
Hybridization. *See* DNA hybridization
Hydrogen sulfide, 2:536
Hydrophilic phospholipids, 1:52
Hydrophobic and hydrophilic, **1:281–282,** 1:351, 2:435
Hydrophobic phospholipids, 1:52
Hydrothermal vents, 1:*282,* **1:282–283,** 2:437, 2:536
Hygiene, **1:283–284**
infection control, 1:310–311
typhoid, 2:558–559
Hyphae, 1:57, 1:230, 1:231, **1:284**
HZV. *See* Herpes zoster virus

I

IEF. *See* Isoelectric focusing
IgA. *See* Immunoglobulins and immunoglobulin deficiency syndromes
IgD. *See* Immunoglobulins and immunoglobulin deficiency syndromes
IgE. *See* Immunoglobulins and immunoglobulin deficiency syndromes
IGF. *See* Insulin-like growth factor
IgG. *See* Immunoglobulins and immunoglobulin deficiency syndromes
IgM. *See* Immunoglobulins and immunoglobulin deficiency syndromes
Illmensee, Karl, 2:658
Immune complex test, **1:285–286**
Immune stimulation, as a vaccine, **1:286**
antiserum and antitoxin, 1:32
T cells (T lymphocytes), 2:539–540
vaccination, 2:567–568
vaccine, 2:568–572
Immune synapse, **1:286–287**
Immune system, **1:287–288**
adjuvant, 1:4–5
AIDS, 1:7–9
allergies, 1:10–11
amino acids in, 1:16
anaphylaxis, 1:17
antigenic mimicry, 1:30–31
autoimmunity and autoimmune diseases, 1:38
B cells (B lymphocytes), 1:43
complement, 1:131
histamine, 1:270–271
histocompatibility, 1:271
human leukocyte antigen (HLA), 1:280
immunosuppressant drugs, 1:306–307
interferons, 1:313–314
miscarriage, 2:483
phagocyte and phagocytosis, 2:434–435
stimulation by external source, 1:286
T cells (T lymphocytes), 2:539–540
See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Immunity; Immunochemistry; Immunodeficiency; Immunologic therapies; Immunology
Immunity, **1:287–291**
active, passive, and delayed, 1:288–290, 1:*289*
cell mediated, 1:290
cytokines, 1:145
globulins, 1:249–250
humoral regulation, 1:290–291
immunosuppressant drugs, 1:306–307
major histocompatibility complex (MHC), 2:361–363
measles, 2:368–369
T cells (T lymphocytes), 2:539–540
See also Immune system; Immunity, active, passive and delayed; Immunity, cell mediated; Immunity, humoral regulation; Immunization; Immunochemistry; Immunologic therapies; Immunology

Immunity, active, passive and delayed, **1:288–290,** 1:*289*
adjuvant, 1:4–5
antiserum and antitoxin, 1:32
B cells, 1:43
opsonization, 2:416–417
phagocyte and phagocytosis, 2:434–435
seroconversion, 2:508
T cells (T lymphocytes), 2:539–540
vaccine, 2:568–572
Immunity, cell mediated, **1:290**
AIDS, 1:9
B cells, 1:43
phagocyte and phagocytosis, 2:434–435
T cells (T lymphocytes), 2:539–540
vaccine, 2:568–572
Immunity, humoral regulation, **1:290–291**
phagocyte and phagocytosis, 2:434–435
T cells (T lymphocytes), 2:539–540
vaccine, 2:568–572
Immunization, **1:291–292**
Behring, Emil von, 1:58–59
immune stimulation, 1:286
phagocyte and phagocytosis, 2:434–435
Salk, Jonas, 2:501–503
See also Vaccination; Vaccine
Immunochemistry, **1:292–293**
Avery, Oswald Theodore, 1:39–41
cytokines, 1:104, 1:145
globulins, 1:249–250
immune system, 1:287–288
major histocompatibility complex (MHC), 2:361–363
reproductive immunology, 2:483–484
seroconversion, 2:508
severe combined immunodeficiency, 2:509–510
T cells (T lymphocytes), 2:539–540
vaccine, 2:568–572
See also Immunity; Immunoglobulins and immunoglobulin deficiency syndromes; Immunologic therapies; Immunological analysis techniques
Immunodeficiency, **1:293–295**
AIDS, 1:7–10
animal models of infection, 1:19
candidiasis, 1:100
human immunodeficiency virus (HIV), 1:279–280
human T-cell leukemia virus (HTLV), 1:281
immunosuppressant drugs, 1:306–307
RNA tumor viruses, 2:493–494
thrush, 2:546–547
See also Immunodeficiency disease syndromes; Immunodeficiency diseases
Immunodeficiency disease syndromes, 1:*295,* **1:295–296**
major histocompatibility complex (MHC), 2:361–363
severe combined immunodeficiency, 2:509–510
See also Immunodeficiency diseases
Immunodeficiency diseases, 1:293
animal models of infection, 1:19
congenital, 1:293
cytokines, 1:104, 1:145
genetic causes, 1:296–297
histocompatibility, 1:271
human leukocyte antigen (HLA), 1:280
major histocompatibility complex (MHC), 2:361–363
mycobacterial infections, atypical, 2:406–407
severe combined immunodeficiency, 2:509–510
toxoplasmosis, 2:548
See also Immunodeficiency disease syndromes
Immunodeficiency diseases, genetic causes, **1:296–297**
Immunoelectrophoresis, 1:182–183, **1:298,** 1:333
See also Electrophoresis
Immunofluorescence, **1:298–299**
bioluminescence, 1:72–73
fluorescent dyes, 1:222
See also Biochemical analysis; Fluorescence; Laboratory techniques in immunology; Laboratory techniques in microbiology
Immunofluorescence microscopy, 1:299
Immunogenetics, **1:299–300**
Berg, Paul, 1:60–62
chemical mutagenesis, 1:114–115
cloning, 1:124
gene, 1:237–238
genetic engineering, 1:74
genotype and phenotype, 1:245–246
major histocompatibility complex (MHC), 2:361–363
oncogene research, 2:415–416
phagocyte and phagocytosis, 2:434–435
radiation mutagenesis, 2:477–478
retroviruses, 2:486–487
severe combined immunodeficiency, 2:509–510
transplantation genetics and immunology, 2:553–554
vaccine, 2:568–572
in vitro and *in vivo* research, 1:307–308
See also Microbial genetics; Molecular biology and molecular genetics
Immunoglobulin deficiency. *See* Immunodeficiency; Immunodeficiency disease syndromes
Immunoglobulins and immunoglobulin deficiency syndromes, **1:300–301**
agammaglobulinemia, 1:293, 1:301
anaphylaxis, 1:17
globulins, 1:249–250
isotypes and allotypes, 1:315–316
opsonization, 2:416–417
severe combined immunodeficiency, 2:509–510
See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Antibody formation and kinetics; Immunodeficiency; Immunodeficiency disease syndromes; Monoclonal antibodies
Immunologic therapies, **1:301–302**
globulins, 1:249–250
immune stimulation, 1:286
immune system, 1:287–288
immunomodulation, 1:305–306
reproductive immunology, 2:483–484
transplantation genetics and immunology, 2:553–554
vaccine, 2:568–572
Immunological analysis techniques, **1:302–303**
enzymes, 1:192–194
major histocompatibility complex (MHC), 2:361–363
Immunological aspects of reproduction. *See* Reproductive immunology
Immunology, **1:303–305**
allergies, 1:10–11
autoimmunity and autoimmune diseases, 1:38
Behring, Emil von, 1:58–59
Berg, Paul, 1:60–62
Centers for Disease Control (CDC), 1:110–112

history of, 1:271–273
immune system, 1:287–288
immunosuppressant drugs, 1:306–307
interferons, 1:313–314
measles, 2:368–369
nutritional aspects, **1:305**
phagocyte and phagocytosis, 2:434–435
radioisotopes, 2:479–480
Schick, Bela, 2:506
transplantation genetics and immunology, 2:553–554
vaccine, 2:568–572
See also Antibody and antigen; Antibody-antigen, biochemical and molecular reactions; Immune system; Immunity; Immunochemistry; Immunogenetics; Immunologic therapies; Laboratory techniques in immunology; Reproductive immunology
Immunology, nutritional aspects, **1:305**
Immunomodulation, **1:305–306**
Immunosuppressant drugs, 1:306–307
Borel, Jean-François, 1:84
thrush, 2:546–547
transplantation genetics and immunology, 2:553–554
In vitro and *in vivo* research, **1:307–308**
Incineration
disposal of infectious microorganisms, 1:161
Indicator species, **1:308,** 2:616–617
Indirect Coombs' test, 2:488
Industrial microbiology. *See* Economic uses and benefits of microorganisms
Infection
animal models of infection, 1:*18,* **1:18–19**
anti-adhesion, 1:23–24
bacteremia, 1:44
chemotherapy, 1:116–117
complement, 1:131
complications of, 1:312–313
defined, 1:309
germ theory of disease, 1:28, 1:246–247, 1:273
immune system, 1:287–288
intracellular, 1:315
invasiveness, 1:315
microbial flora of the oral cavity, dental caries, 2:379–380
microbial flora of the skin, 2:380
microbial flora of the stomach and gastrointestinal tract, 2:380–381
nosocomial infection, 2:411–412
pertussis, 2:429–430
phagocyte and phagocytosis, 2:434–435
prions, 2:449
skin infections, 2:516–517
sulfa drugs, 2:535
symptoms, 1:312
See also Bacterial infection; Fungal infection; Infection and resistance; Infection control; Viral infection
Infection and resistance, **1:308–310,** 1:*309*
invasiveness and intracellular infection, 1:315
See also Bacterial infection; Fungal infection; Infection control; Viral infection
Infection control, **1:310–311,** 1:*311*
antiseptics, 1:31–32
chlorination, 1:119–120
hygiene, 1:310–311
microbial flora of the oral cavity, dental caries, 2:379–380
microbial flora of the skin, 2:380
microbial flora of the stomach and gastrointestinal tract, 2:380–381
Pasteur, Louis, 2:424–426
steam pressure sterilizer, 2:530–531
sterilization, 1:31, 1:154, 1:158, 2:530, 2:531–532, 2:546
See also Epidemiology; Infection; Infection and resistance
Infection hypothesis, 1:31
Infectious mononucleosis, 1:201, **2:399**
Inflammation, **1:311,** 1:*311*
autoimmunity, 1:38
histamine and, 1:16
phagocyte and phagocytosis, 2:434–435
Influenza, **1:311–313,** 1:*312*
epidemics, 1:193–194, 1:196–198, 1:312
Great Flu Epidemic of 1918, 1:193, 1:220–221
history of, 1:193
Influenza virus, 1:262, 1:312, 1:*312,* 2:570
Ingenhousz, Jan, 2:645
Inhalation anthrax, 1:70, 1:76
Inherent resistance, 1:47
Inoculating loop, 1:335
Insecticides, *Bacillus thuringiensis,* 1:43–44, 1:*44*
Insertion mutations, 2:405, 2:406
Insertion sequences, 1:200, 2:486
The Institute for Genomic Research (TIGR), 1:49, **2:544**
Insulin-like growth factor (IGF), 1:104, 1:106
Interferon actions, **1:313,** 2:568–572
Interferon-alpha, 1:314
Interferon-gamma, 1:314
Interferon-tau, 1:314
Interferons, 1:313, **1:313–314,** 2:568–572
Interleukin-2, 1:233, 1:234
Intermediate yellow fever, 2:613
Interphase, 1:103, 1:243
Intracellular infection, 1:315
Introns, 2:457
Invasiveness and intracellular infection, **1:315**
immune system, 1:287–288
virus replication, 2:581–582
Iridovirisae, 2:536
Irish potato famine, 1:231
"Iron" bacteria, 1:115, 2:514
Isaacs, Alick, 1:314, 2:656
Isayev, Vasily Isayevich, 2:432
Iso-Sensitest agar, 1:23
Isoelectric focusing (IEF), 1:183
Isotypes and allotypes, **1:315–316**
Ivanovsky, Dmitri Iosifovich, **1:316,** 2:528, 2:650

J

Jacob, François, 1:87, 1:141, **1:317–319,** 1:*318,* 2:375, 2:381, 2:398, 2:655, 2:656
Jacobs, William, 2:660
Jannasch, Holger Windekilde, 1:283, **1:319,** 2:658
Janssen, Hans, 2:644
Janssen, Zacharias, 2:644
Japanese B encephalitis, 2:499
JC papovirus, 2:519
Jeffreys, Alex, 2:658
Jenner, Edward, 1:28, 1:32, 1:196, 1:246, 1:271, 1:272, 1:274, 1:287, 1:292, 1:303, **1:319–321,** 1:*320,* 2:521, 2:569, 2:584, 2:646

Jerne, Niels D., **1:321–322,** 2:392
Jock itch, 2:517
Johannsen, Wilhelm, 1:237, 2:651
Johnsen, C.D., 1:252
Johnson, Claude D., 2:402
Johnson, Irving S., 1:116
Johnson, William Arthur, 1:331
"Jumping genes," 2:555
Jungle yellow fever, 2:613
Junin virus, 1:34, 1:35
Justinian, 2:644

K

Kahn, Reuben Leon, 2:538
Kaposi's sarcoma, 1:267, 1:314
Karstroem, Henning, 2:398
Karyotype analysis, 1:121, 2:415
Kearns-Sayre syndrome, 2:393
Kelp and kelp forests, 1:6, **1:323–324,** 2:421, 2:460
Kendall, Edward, 1:287
Keratitis, 1:212
Keratoconjunctivitis, 1:3
Khorana, Har Gobind, **1:324–325,** 2:657
Killer T cells, 1:288
Kinetochores, 1:244
Kirby-Bauer disk-diffusion assay, 1:7
"Kissing bugs," 1:111
"Kissing disease." *See* Mononucleosis, infectious
Kitasato, Shibasaburo, 1:59, 1:272, 1:287, **1:325**
Klebs, Edward Theodore, 2:650
Klebsiella, ultrastructure of, 1:*52*
Klebsiella infection, 1:188
Kluyver, Albert Jan, **1:325–326,** 2:409, 2:652
Knoll, Max, 1:179–180, 2:496
Knoop, Franz, 1:330
Koch, Robert, 1:59, 1:177, 1:252, 1:272, 1:273, 1:*274,* 1:325, **1:326–328,** 1:*327,* 1:353, 2:648, 2:649, 2:650
  agar, 1:6
  anthrax, 1:19, 1:247, 1:326
  cholera, 1:327
  Pfeiffer, Richard, 2:432
  tuberculosis, 1:327, 2:555
Koch's postulates, 1:247, 1:274, 1:327, **1:328,** 1:353
Kohl, 1:31
Köhler, Georges, 1:28, 1:30, 1:321, **1:328–329,** 2:392, 2:657
Kolletschka, Jakob, 2:508
Koplik's sports, 2:368
Kornberg, Arthur, 1:324, 2:655, 2:656
Kossel, Albrecht, 1:162, 2:489
Kovalevsky, Alexander, 2:377
Krebs, Hans Adolf, 1:192, **1:329–331,** 2:653
Krebs cycle, **1:331–332,** 2:392–393
Kristensen, Reinhardt Mobert, 2:659
Kühne, Wilhelm Friedrich, 2:649
Kunkel, Henry, 1:175, 1:176
Kuru, 1:90
Kyasanur Forest disease, 1:263

L

L-forms, 2:462
Laboratory techniques in immunology, **1:333–334,** 1:*334,* 2:*439*
  contamination, 1:133–135
  disposal of infectious microorganisms, 1:160–161
  electrophoresis, 1:182–183, 1:333
  ELISA, 1:190–191, 1:333–334
  enzyme-linked immunosorbant assay (ELISA), 1:190–191
  epidemiological tools, 1:199–200
  fluorescence *in situ* hybridization (FISH), 1:221–222, 2:415
  growth and growth media, 1:254–255, 1:335–336
  immune complex test, 1:285–286
  immunoelectrophoresis, 1:298, 1:333
  Koch's postulates, 1:247, 1:274, 1:327, 1:328, 1:353
  monoclonal antibodies, 1:334
  pipette, 2:438–439
  protein crystallography, 2:452
  Pyrex, 2:468–469
  recombinant DNA molecules, 2:480–481
  spectroscopy, 2:524–525
  in vitro and *in vivo* research, 1:307–308
  See also Biochemical analysis techniques; Immunological analysis techniques; Microscope and microscopy
Laboratory techniques in microbiology, 1:*335,* **1:335–336,** 2:*439*
  acridine orange and, 1:2–3, 1:24
  agar and agarose, 1:5–7
  agar diffusion, 1:7
  antibodies, 1:27
  bacteriophages and bacteriophage typing, 1:55–56
  blood agar, hemolysis, and hemolytic reactions, 1:80
  buffer, 1:95–96
  conjugation, 1:133–134
  contamination, 1:133–135
  Coulter counter, 1:137–138
  culture, 1:144–145
  dilution theory and techniques, 1:156
  disposal of infectious microorganisms, 1:160–161
  electron microscopic examination of microorganisms, 1:180–181
  epidemiological tools, 1:199–200
  fluorescence *in situ* hybridization (FISH), 1:221–222, 2:415
  germ theory of disease, 1:28, 1:246–247, 1:273
  Gram staining, 1:253–254
  growth and growth media, 1:254–255, 1:335–336
  Koch's postulates, 1:247, 1:274, 1:327, 1:328, 1:353
  Petri, Richard Julius, 2:430–431
  pipette, 2:438–439
  polymerase chain reaction (PCR), 2:446–447
  protein crystallography, 2:452
  Pyrex, 2:468–469
  radioisotopes, 2:479–480
  recombinant DNA molecules, 2:480–481
  spectrophotometer, 2:523–524
  spectroscopy, 2:524–525
  steam pressure sterilizer, 2:530–531
  sterilization, 2:531–532
  thermal death, 2:546
  in vitro and *in vivo* research, 1:307–308
  See also Biochemical analysis techniques; Microscope and microscopy
LAC. *See* Lupus anticoagulant
Lactic acid bacteria, **1:336,** 2:381
Lactobacillus, 1:23, **1:336–337**
Lactobacillus acidophilus, 1:174, 1:337, 2:450
Lactobacillus bulgaricus, 1:337
Lactobacillus GG, 1:337, 2:450
LAD. *See* Leukocyte adhesion defect

Lamarck, Jean, 2:646
Lambda pipette, 2:439
Lancefield, Donald, 1:337
Lancefield, Rebecca Craighill, **1:337–338**
Landsteiner, Karl, 1:29, 1:287, **1:338–340,** 1:*339,* 2:509, 2:651
Laplace, Pierre-Simon, 2:646
Lassa fever, 1:34, 1:263
Lassa virus, 1:34
Latent viruses and disease, **1:340–341**
  Epstein-Barr virus, 1:82, 1:201, 1:267, 2:399
  herpes and herpes virus, 1:267–268
  varicella zoster virus, 2:573–574
LAV. *See* Lymphadenopathy-associated virus
Laveran, C.L. Alphonse, 2:649
Lavoisier, Antoine-Laurent, 2:645, 2:646
LCR. *See* Ligase change reaction
Leber hereditary optic neuropathy (LHON), 2:393
Lederberg, Esther M., 2:654, 2:655
Lederberg, Joshua, 1:55, 1:133, 1:274, **1:341–343,** 1:*342,* 2:382, 2:541, 2:542, 2:654, 2:655, 2:656
Leeches, 2:423
Leeuwenhoek, Anton van, 1:1, 1:246, 1:273, 1:*343,* **1:343–344,** 2:388, 2:645
Legionella pneumophila, 2:445
  invasiveness of, 1:315
  Legionnaires' disease, 1:344
  Pontiac disease, 1:345
Legionnaires' disease, **1:344–346,** 1:*345*
Leishmania, 2:423
Lenscrafting, Abbe, Ernst, 1:1
Lentivirinae, 2:493
Lentiviruses, 2:519
Lepromatous leprosy, 1:346–347
Leprosy, 1:196, 1:251, 1:*309,* **1:346–348,** 1:*347*
Leptothrix discophora, 2:514
Lethal dose 50, 1:336
Leukemia virus. *See* Human T-cell leukemia virus
Leukocyte adhesion defect (LAD), 1:297
Levaditi, Constantin, 1:340
Levan, Albert, 2:656
Levene, Phoebus, 2:489, 2:651, 2:653
Levine, Philip, 1:339, 1:340
LHON. *See* Leber hereditary optic neuropathy
Lice, 2:423
Lichen planus, **1:348**
Lichens, **1:348–349,** 2:407, 2:411
Life, origin of, **1:349–351,** 1:*350*
  Murchison meteorite, 2:403
  oxygen cycle, 2:418–419
Ligase change reaction (LCR), 1:240
Light microscope, 2:388, 2:*389*
Lindenmann, Jean, 1:314, 2:656
LINEs. *See* Long interspersed elements
Linkage mapping, 1:241–242
Linnaeus, Carolus, 2:450, 2:645
Lipmann, Fritz, 1:331, 2:653
Lipopolysaccharide and its constituents, 1:47, **1:351–352**
Lister, Joseph, 1:134, 1:247, 1:274, **1:352,** 1:*353,* 2:411
Listeria monocytogenes, 2:466
Listeriosis, 2:466
Localized mutagenesis, 2:406
Lockjaw. *See* Tetanus
Loeb, Jacques, 2:651
Loeffler, Friedrich August Johannes, 1:227, **1:352–354,** 1:*353,* 2:650, 2:651
Loefflerella mallei, 1:353
Long interspersed elements (LINEs), 2:486
Long terminal repeats (LTRs), 2:486
LPS. *See* Lipopolysaccharide
LTRs. *See* Long terminal repeats
Luciferase, 1:72, 1:73, 1:354
Luciferase reaction, 1:73
Luciferin, 1:72, 1:73
Lucretius, 2:643
Ludwig, Carl Friedrich Wilhelm, 1:255
Ludwig, Ernst, 1:339
Luminescent bacteria, **1:354,** 2:474
Lupus anticoagulant (LAC), 2:483
Luria, Salvador E., 1:55, 1:268, 1:269, 2:594, 2:653, 2:654, 2:657
lux gene system, 1:354
LuxR, 1:354
Lwoff, André, 1:317, 2:397, 2:398, 2:656
Lyme disease, 1:82, 1:123, **1:355–356,** 1:356, 2:468
Lymphadenopathy-associated virus (LAV), 2:400
Lymphocytes. *See* T cells (T lymphocytes)
Lymphokines, 1:291
Lysogeny, 1:340–341, **1:356–357,** 2:434
Lysosomes, 1:146, **1:357**

M

M protein, 1:338
Machupo virus, 1:34
MacLeod, Colin Munro, 1:114, 1:274, **2:359–360,** 2:367, 2:654
MacPherson, Ian, 2:400
Macrocystis pyrifera, 1:323
Macrophages, 1:315
Mad cow disease. *See* BSE and CJD disease
Madei, 2:519
Maedi-visna virus, 2:519
Magnetospirillum magnetotacticum, 2:360, 2:361
Magnetotactic bacteria, 1:63, **2:360–361,** 2:525–526
Mahoney, John F., 2:538
Major histocompatibility complex (MHC), 1:292, **2:361–363,** 2:554
  autoimmunity, 1:38
  histocompatibility, 1:271
  human leukocyte antigens (HLAs), 1:280
  transplantation genetics and immunology, 2:553–554
Malaria and physiology of parasitic infections, **2:363–364,** 2:443, 2:461
Mallon, Mary, 2:559
Malphigi, Marcello, 2:645
MALT. *See* Mucus-associated lymphoid tissue
"Malta" fever, 1:206
Mamamorosch, Karl, 2:655
Mantoux test, 1:290
Marburg hemorrhagic fever, 1:263
Marburg virus, 1:264, 2:585
Margulis, Lynn, **2:364–365,** 2:657
Marine microbiology, 2:*365,* **2:365–366**
  bioluminescence, 1:72–73, 1:354
  Jannasch, Holger Windekilde, 1:319
  kelp and kelp forests, 1:323–324
Marrack, John, 2:653
Mars
  bacterial fossils in Martian rocks, 1:228

extraterrestrial microbiology, 1:209–210, 1:*210,* 1:228
magnetotactic bacteria, 2:361
Marshall, Barry J., 1:123, **2:366**
Marshall, Richard E., 2:657
Mast cells, 1:17
Mastigophora, **2:366–367**
Matin, A.C., **2:367**
Matthaei, Heinrich, 1:238
Mayer, Adolf, 2:540
McCarty, Maclyn, 1:114, 1:274, 2:359, **2:367–368,** 2:654
McClintok, Barbara, 2:555, 2:653, 2:654
McDermott, Katherine, 2:653
McKie, Margot, 2:652
McMaster, P.D., 2:494
MCPs. *See* Methyl-accepting chemotaxis proteins
MDR TB. *See* Multidrug-resistant tuberculosis
Measles, **2:368–369,** 2:*369*
Measles, mumps, and rubella (MMR) vaccine, 2:567
Measles vaccine, 2:369
Measles virus, 2:368, 2:519
Medawar, Peter Brian, 1:96, 1:288, 1:306–307, **2:369–371**
Medical training and careers in microbiology, **2:371–373,** 2:*372*
Meiosis, 1:104–105, 2:609
Meiosis I, 1:104–105
Meiosis II, 1:105
Melanin, 1:16
MELAS. *See* Mitochondrial encephalomyopathy, lactic acidosis and strokelike episodes
Melatonin, 1:16
Membrane fluidity, **2:373**
hydrophobic and hydrophilic, 1:281–282
phospholipids, 2:435
Membrane transport. *See* Cell membrane transport; Prokaryotic membrane transport
Mendel, Gregor, 1:237, 2:647, 2:648
Meningitis, bacterial and viral, **2:374–375**
cryptococcal, 1:142, 1:143
enterovirus-induced, 1:190
epidemics, 1:195–196
Menten, Maud, 1:192
6-Mercaptopurine, 1:117, 1:184
MERFF. *See* Myoclonus epilepsy with ragged red fibers
Meselson, Matthew Stanley, 1:87, **2:375–376**, 2:656
Mesocyclops, 2:616
Mesophilic bacteria, 1:50, **1:276**
Mesophyll, 1:120
Messenger RNA (mRNA), 2:489, 2:491, 2:548, 2:551–552
Metabolism, **2:376–377**
aerobes, 1:5
carbon cycle, 1:101
chloroplast, 1:119, 1:120
enzyme induction and repression, 1:191–192
mitochondria, 2:392–393
nitrogen cycle, 2:410–411
oxidation-reduction reaction, 2:417
oxygen, 2:418–419
photosynthesis, 2:436
sulfur cycle, 2:536
Metaphase, 1:103, 1:243
Metaphase I, 1:105
Metaphase plate, 1:107
Metchnikoff, Élie, 1:83, 1:178, 1:272, 1:303, **2:377–378,** 2:434, 2:450, 2:650
Methane oxidizing and producing bacteria, 1:101, **2:378–379,** 2:601
Methanococcus jannaschii, 2:378–379, 2:659
Methanotrophs, 1:101
Methicillin-resistant *Staphylococcus aureus* (MRSA), 2:529
Methyl-accepting chemotaxis proteins (MCPs), 1:37
Meyerhof, O., 1:217
MHC. *See* Major histocompatibility complex
Michaelis, Leonor, 1:192
Michel, Hartmut, 2:436
Microaerophilic organisms, 1:5
Microarrays, 1:164
See also DNA chips and microarrays
Microbial contamination. *See* Contamination
Microbial flora of the oral cavity, dental caries, **2:379–380**
Actinomyces, 1:3
plaque, 1:17, 1:67, 2:387, 2:442
thrush, 2:546–547
Microbial flora of the skin, **2:380,** 2:383
acne, 1:1–2
coryneform bacteria, 1:136–137
Microbial flora of the stomach and gastrointestinal tract, **2:380–381**
Enterobacteriaceae, 1:187
helicobacteriosis, 1:262
Lactobacillus, 1:336–337
probiotics, 2:450
pseudomembranous colitis, 2:465
Microbial genetics, **2:381–382**
cladistics, 2:438
cloning, 1:124
enhanced tolerance or sensitivity to temperature and pH ranges, 2:404–405
enzyme induction and repression, 1:191–192
episomes, plasmids, insertion sequences, and transposons, 1:200
fungal genetics, 1:230–231
gene, 1:237–238
genetic identification of microorganisms, 1:240–241
genetic mapping, 1:241–242
genotype and phenotype, 1:245–246
heat shock response, 1:261
Neurospora, 2:409–410
operon, 1:237, 1:354, 2:398, 2:416
phage genetics, 2:433–434
phage therapy, 2:434
phylogeny, 2:437–438
recombination, 2:481
retroposons and transposable elements, 2:485–486
transcription, 2:548–549
transduction, 2:549
transformation, 2:549–550
transgenics, 2:550–551
translation, 2:551–553
transplantation genetics and immunology, 2:553–554
transposition, 2:554–555
See also DNA; Genetic identification of microorganisms; Immunogenetics; Molecular biology and molecular genetics; Phage genetics; RNA
Microbial symbiosis, **2:382–383**
lichens, 2:382–383, 2:407, 2:411
nitrogen-fixing bacteria, 2:411
soil formation, involvement of microorganisms, 2:523
Microbial taxonomy, 2:382, **2:383–384**
Microbiology
careers in, 2:371–373, 2:*385,* 2:*386*

clinical, 2:384–387
historical chronology, 2:643–660
history of, 1:273–274
medical training and careers in microbiology, 2:371–373
petroleum microbiology, 2:431–432
proteomics, 2:457–458
radioisotopes, 2:479–480
in vitro and *in vivo* research, 1:307–308
See also History of microbiology; Laboratory techniques in microbiology; Marine microbiology; Microscope and microscopy; Qualitative and quantitative analysis in microbiology; Quality control in microbiology; Veterinary microbiology
Microbiology, clinical, **2:384–387,** 2:*385,* 2:*386*
Microcystin, 1:82
Microcystis aeruginosa, 1:82
Microorganisms, **2:387**
attractants and repellents, 1:37
biogeochemical cycles, 1:68–69
carbon cycle, 1:100–101
nitrogen cycle, 2:410–411
oxygen cycle, 2:418–419
sulfur cycle, 2:536
See also Bacteria; Bacterial infection; Fungal infection; Fungi; Genetic identification of microorganisms; Microbial symbiosis; Microbial taxonomy; Microscope and microscopy; Viral infection; Viruses and responses to viral infection
Micropipettes, 2:439
Microscope and microscopy, 1:335, **2:388–389,** 2:*389*
Abbe, Ernst, 1:1
atomic force microscope, 1:36–37
bacterial ultrastructure, 1:53–54
electron microscope, 1:179–180
electron microscopic examination of microorganisms, 1:180–181
epifluorescence microscopy, 1:222
field ion microscope, 1:180
fluorescent dyes, 1:222
immunofluorescence microscopy, 1:299
light microscope, 2:388, 2:*389*
negative staining, 1:181
scanning confocal microscope, 2:473
scanning electron microscopy (SEM), 1:180, 2:388
scanning tunneling microscope (STM), 2:388
spectroscopy, 2:524–525
transmission electron microscope (TEM), 1:*179,* 1:179–181, 2:388
See also Dyes; Laboratory techniques in microbiology
Microwave spectroscopy, 2:524
Miescher, Johann, 1:161, 2:488, 2:648
Miller, Jacques, 2:656
Miller, Stanley L., 1:351, 2:389, **2:390–391**
Miller-Urey experiment, **2:389–390,** 2:403, 2:563
Milstein, César, 1:28, 1:30, 1:321, **2:391–392,** 2:*392,* 2:657
Milstein-Köhler technique, 1:30
Missense mutations, 2:405
Mitchell, Peter, 1:182
Mites, 2:423
Mitochondria and cellular energy, **2:392–393**
disorders of, 2:393
Krebs cycle, 1:331–332
mitochondrial DNA, 2:393
mitochondrial inheritance, 2:393–394
Mitochondrial DNA, **2:393**
mitochondria and cellular energy, 2:392–393
mitochondrial inheritance, 2:393–394
See also Mutations and mutagenesis
Mitochondrial encephalomyopathy, lactic acidosis and strokelike episodes (MELAS), 2:393
Mitochondrial EVE, 2:394
Mitochondrial inheritance, **2:393–394**
Miller-Urey experiment, 2:389–390, 2:403
mitochondria and cellular energy, 2:392–393
mitochondrial DNA, 2:393
See also Mutations and mutagenesis
Mitosis, 1:103–104, 1:*104*
eukaryotes, 1:106–107, 1:121, 1:243–244
MMR. *See* Measles, mumps, and rubella (MMR) vaccine
Moist heat sterilization, 2:532
Mold, **2:394–395**
colony and colony formation, 1:129–130
Dictyostelium discoideum, 1:155
eye infections, 1:213
Neurospora crassa, 2:409–410
Sick Building Syndrome, 2:408
slime molds, 1:155, 2:461, 2:518–519
See also Mycology
Molecular biology and molecular genetics, **2:395–397,** 2:*396*
amino acid chemistry, 1:14–16, 1:*15*
Asilomar conference, 1:36
bacterial artificial chromosome (BAC), 1:48–49
bacterial ultrastructure, 1:53–54
fluorescence *in situ* hybridization (FISH), 1:221–222, 2:415
gene, 1:237–238
mitochondrial inheritance, 2:393–394
oncogene, 2:415
phenotype and phenotypic variation, 2:435
plasmids, 1:200, 2:442–443
polymerase chain reaction (PCR), 2:446–447
protein crystallography, 2:452
protein export, 2:453–454
proteomics, 2:457–458
radiation mutagenesis, 2:477–478
restriction enzymes, 2:485
transduction, 2:549
transformation, 2:549–550
transgenics, 2:550–551
translation, 2:551–553
in vitro and *in vivo* research, 1:307–308
See also Mutations and mutagenesis
Molecular chaperones, 1:113
Molecular cloning, 1:75
Molecular rotational resonance spectroscopy, 2:524
Möllendorff, Wilhelm von, 1:330
Monera, 2:450
Monoclonal antibodies, 1:28, **1:29–30,** 1:304, 1:334
Monod, Jacques Lucien, 1:138, 1:141, 1:318, 2:381, **2:397–399,** 2:656
Mononucleosis, infectious, 1:201, **2:399**
Monovalent antiserum, 1:32
Montagnier, Luc, 1:7, 1:233, 1:234, **2:399–401,** 2:*400,* 2:658
Montague, Mary Wortley, 1:246, **2:401–402,** 2:569
Moore, Ruth Ella, **2:402**
Moore, Stanford, 2:657
Morgan, Thomas Hunt, 1:161, 1:237, 1:241, 2:398, 2:652
Mosquitoes, as carriers of disease, 2:423
Mössbauer, Rudolf, 2:525
Mössbauer effect, 2:525

Mössbauer spectroscopy, 2:525
Most probable number method, 1:156
Motility, 1:52, 1:249, 2:473
See also Bacterial movement
Mouth. *See* Microbial flora of the oral cavity, dental caries
mRNA. *See* Messenger RNA
MRSA. *See* Methicillin-resistant *Staphylococcus aureus*
Mucor, 2:394
Mucus-associated lymphoid tissue (MALT), 1:290
Muller, Erwin Wilhelm, 1:180
Muller, Hermann Joseph, 2:652
Mullis, Kary, 2:658
Multidrug-resistant tuberculosis (MDR TB), 2:556
Multiplex PCR, 1:240–241
Mumps, **2:402–403**
Mumps virus, 2:402
Murchison meteorite, **2:403**
Murein. *See* Peptidoglycan
Murine typhus, 2:560
Murray, Andrew W., 2:658
Murray, Robert, **2:403–404,** 2:478
Mushrooms, 1:57, 1:117, 1:232
Mutants: enhanced tolerance or sensitivity to temperature and pH ranges, **2:404–405,** 2:433
Mutations and mutagenesis, 1:207, 2:384, **2:405–406**
chemical mutagenesis, 1:114–115
hemagglutinin(HA) and neuraminidase (NA), 1:262–263
immunogenetics, 1:28, 1:299–300
mutants: enhanced tolerance or sensitivity to temperature and pH ranges, 2:404–405, 2:433
oncogene research, 2:415–416
proteins, 1:15
radiation and, 2:477–478
See also Microbial genetics
Mutualism, 2:382, 2:383
Mycelium, 1:230, 1:231, 2:394, **2:406**
Mycobacterial infections, atypical, **2:406–407**
Mycobacterium avium, 1:347
Mycobacterium leprae, 1:108, 1:346–348
Mycobacterium tuberculosis, 1:123, 2:555, 2:660
Mycology, **2:407–408**
See also Fungal genetics; Fungal infection; Fungi; Fungicides; Lichens; Mold; Yeast
Mycoplasma, 1:52, 2:576
Mycoplasma fermentans, 2:408
Mycoplasma genitalium, 2:408, 2:659
Mycoplasma infections, **2:408**
Mycoplasma pneumoniae, 2:408
Mycorrhizae, 1:57
Mycotoxins, 2:394, 2:395
Myeloma, 1:304
Myoclonus epilepsy with ragged red fibers (MERFF), 2:393
Mysidacea, 2:616
Myxobacteria, 1:249
Myxoma virus, 2:507
Myxomycota, 2:518

N

N protein, 2:433
Nageli, Carl Wilhelm von, 1:255
"Naked" DNA, 1:10
National Center for Human Genome Research (NCHGR), 2:660
National Electronic Data Surveillance System (NEDSS), 1:79
National Human Genome Research Institute (NHGRI), 2:660
National Pharmaceutical Stockpile Program (NPS), 1:78
Natural resistance, 1:47
Natural selection, 1:208, 2:506–507
NCHGR. *See* National Center for Human Genome Research
Necrotizing enterocolitis, 1:188
Necrotizing fasciitis, 2:534
NEDSS. *See* National Electronic Data Surveillance System
Negative staining, 1:181
Neisser, Albert, 1:251
Neiserria, eye infections, 1:213
Neiserria gonorrheae, 1:48
Neisseria meningitides, 1:22, 1:195
Nematodes, 2:423
Nereocystis leutkeana, 1:323
Neuberg, Carl, 2:651
Neuraminidase (NA), 1:263
Neuroritinitis, 1:212
Neurospora, **2:409–410**
Neurospora crassa, 1:230, 2:409, 2:541
Neurotoxins
Clostridium tetani, 2:543
Pyrrophyta, 2:470
Neurotransmitters, 1:16
Neva, Franklin Allen, 2:424, 2:597
NHGRI. *See* National Human Genome Research Institute
Nicolle, Charles-Jean-Henri, 2:402
Nicolson, G.L., 2:373
Nikaido, Hiroshi, 2:447
Nirenberg, Marshall Warren, 1:141, 1:238, 2:656, 2:657
Nisser, Albert, 2:649
Nitrate, 2:410, 2:411
Nitrifying bacteria, 1:115
Nitrobacter, 1:115, 2:411
Nitrogen cycle in microorganisms, **2:410–411**
Azotobacter, 1:41
biogeochemical cycles, 1:68–69
Nitrogen fixation, *Azotobacter,* 1:41
Nitrogen-fixing bacteria, 2:410
Nitrogenase, 2:410
Nitrosomonas, 1:115, 2:411
Nitzchia occidentalis, 2:482
NMR. *See* Nuclear magnetic resonance
Noctiluca, 1:156
Non-culturable bacteria. *See* Viable but non-culturable bacteria
Non-specific immunity. *See* Immunity, active, passive and delayed
Nonsense mutations, 2:405
Nontyphoidal *Salmonella* infection, 1:188
Nori, 2:488
North Asian tick typhus, 2:493
Northern blotting, 1:183
Northrop, John N., 1:192, 2:654
Norwalk virus, gastroenteritis, 1:236
Nosocomial infections, **2:411–412,** 2:*412*
Notobiotic animals. *See* Animal models of infection
Novotny, Ergo, 2:*453*
NPS. *See* National Pharmaceutical Stockpile Program
Nuclear magnetic resonance (NMR), 2:524
Nucleic acids, 1:238–240, 2:488
See also DNA; RNA
Nuclein, 2:489
Nucleolus, 2:412

Nucleotides, 2:488, 2:552
See also Genetic code
Nucleus, **2:412,** 2:*413*
Nutrition, immunology and, 1:305
Nuttall, George H.F., **2:413–414**
Nystatin, 1:261

O

Ochoa, Severo, 1:324, 2:656
O157:H7, infection. *See Escherichia coli*
Oil spills, 2:*431*
bioremediation, 1:73, 1:*74,* 2:431–432
Olitsky, Peter K., 2:500
Olson, Maynard, 2:658
OmpC, 2:447
OmpF, 2:447
Omsk hemorrhagic fever, 1:263
Oncogene, 1:104, 1:243, **2:415,** 2:480, 2:558
See also Oncogene research
Oncogene research, 1:299–300, **2:415–416,** 2:558
See also Oncogene
Oncovirinae, 2:493
Oomycota, 2:461
Oparin, Aleksander, 1:350
Operating rooms, infection control, 1:310
Operon, 1:237, 1:354, 2:398, **2:416**
Opossum shrimp, 2:616
Opportunistic infections, 2:612
See also Nosocomial infections
Opsonization, 1:131, **2:416–417**
Optic infections. *See* Eye infections
Oral cavity. *See* Microbial flora of the oral cavity
Origin of life. *See* Life, origin of
Orla-Jensen, Sigurd, 2:651
Oropharyngeal candidiasis, 2:546
Orthohepadnavirus, 1:264
Orthomyxoviruses, 1:311–312, 2:580, 2:584
Oscillatoria, 1:52
Osmosis, cell membrane transport, 1:109
Oswald-Folin pipette, 2:439
Otic infections. *See* Ear infections
Otitis media, 1:172
Ottenberg, Reuben, 1:339
Owen, Ray D., 2:370
Oxidation-reduction reaction, **2:417**
Oxygen cycle in microorganisms, 1:68–69, 2:*418,* **2:418–419,** 2:437
Oxyluciferin, 1:72
Oysters, toxins in, 1:226, 1:*226*

P

Pandemics. *See* Epidemics and pandemics
Panos, Theodore Constantine, 2:424
Papillomavirus, 2:513
Papovaviruses, 2:581, 2:584
Paracelsus, 2:644
Parainfluenzae virus, 2:575
Paralytic polio, 2:446
Paralytic rabies, 2:476
Paralytic shellfish poisoning, 1:157, 2:482
Paramecium, **2:421–422,** 2:*422,* 2:459, 2:463
Paramyxovirus group, 2:368
Parasexual systems, 1:230
Parasites, **2:422–423**
Entamoeba histolytica, 1:11, 1:12, 1:169, 1:186–187, 1:315
Giardia, 1:248–249
hyphae, 1:284
life cycle of, 2:363
mastigophora, 2:366–367
Plasmodium, 2:363, 2:443–444
protozoa, 2:462–464
rare genotype advantage, 2:480
Sporozoa, 2:459, 2:526
Parasitic infection, 2:423–424
amebic dysentery, 1:11–12, 1:169, 1:186–187, 1:248–249, 2:423
Chagas disease, 1:111–112
cryptosporidiosis, 1:143
cryptosporidium, 1:143
giardiasis, 1:248–249
malaria, 2:363–364
toxoplasmosis, 2:548
Parasitism, 2:382
Parasitology, 2:422
Pardée, Arthur, 1:141, 1:318
Park, James T., 2:655
Parkman, Paul Douglas, **2:424**
Parvoviruses, 2:580, 2:584
Passive immunity, 1:288–290
Passive immunization, 1:289–290
Pasteur Institute, 2:426, 2:650
Pasteur pipette, 2:439
Pasteur, Louis, 1:*18,* 1:167, 1:192, 1:*247,* 1:303, **2:424–426,** 2:*425*
animal models of infection, 1:18
anthrax, 2:425
fermentation, 2:647
food preservation, 1:224
germ theory of disease, 1:28, 1:246–247, 1:273
pasteurization, 1:54, 1:246, 1:272, 2:426–427, 2:532, 2:569
rabies, 2:425, 2:475, 2:650
vaccines, 1:289, 2:495–496, 2:569
Pasteurella, 2:426, **2:426**
Pasteurella multocida, 1:23, 2:426, 2:576
Pasteurella pneumotrophica, 2:426
Pasteurization, 1:54, 1:246, 1:272, **2:426–427,** 2:532, 2:569
Pathogens. *See* Microbiology, clinical; Transmission of pathogens
PBPs. *See* Penicillin-binding proteins
PCR. *See* Polymerase chain reaction
PDGF. *See* Platelet-derived growth factor
Pearson, Karl, 2:651
Pelagophycus porra, 1:323
Penicillin, 1:25, **2:427–429**
bactericidal nature of, 1:54–55
Fleming, Alexander, 1:218–219
history of, 1:112, 1:276, 2:511
Streptococcus, 2:533
Penicillin-binding proteins (PBPs), 2:427
Penicillin F, 1:276
Penicillin G, 1:276
Penicillin V, 1:276
Penicillium camemberti, 2:395
Penicillium chrysogenum, 1:230
Penicillium mold, 2:395
Penicillium notatum, colony, 1:*130,* 1:*277*
Penicillium roqueforti, 2:395
Penninger, Josef Martin, 1:123, **2:428**

Peptidoglycan, 1:25, 1:51, 2:427, 2:428, **2:429**
Peptostreptococcus, 1:16
Periplasm, 1:51, **2:429,** 2:453
Perlmann, Gertrude, 1:338
Perry, Seymour, 1:233
Pertussis, **2:429–430**
Pesticide resistance, 2:506
Pestivirus, 2:536
Petri, Richard Julius, **2:430–431,** 2:650
Petri dish (Petri plate), 1:335, 2:430
Petroleum microbiology, **2:431–432,** 2:488
Petroleum spills, bioremediation, 1:73
Pettenkoffer, Max Josef von, 1:252, 1:255
Pfeiffer, Richard Friedrich Johannes, 1:83, 1:287, **2:432–433**
Pfeifferella, 2:432
Pfeiffer's agar, 2:432
Pfeiffer's phenomenon, 2:432
pH, 1:95–96, **2:433**
pH sensitivity. *See* Mutants: enhanced tolerance or sensitivity to temperature and pH ranges
Phaeophyta, 1:323–324, 2:421, **2:421,** 2:460
Phage genetics, **2:433–434**
  radiation mutagenesis, 2:477–478
  See also Bacteriophage and bacteriophage typing
Phage therapy, **2:434**
  See also Bacteriophage and bacteriophage typing
Phagocyte and phagocytosis, 1:5, **2:434–435**
Phagocyte defects. *See* Immunodeficiency disease syndromes
Phagocytosis, 1:5, 1:109, 2:434
  bacterial surface layers, 1:53
  defined, 1:291
  opsonization, 2:416–417
Phase G0, 1:103
Phenol oxidase, 1:142–143
Phenotype and phenotypic variation, 1:101, 1:208, 1:245, **2:435**
  See also Genotype and phenotype
Phi X 174, 2:516
Phosphodiester, 1:120
Phosphoglycerides, 2:435
Phospholipids, 1:52, **2:435**
Photoautotrophic organisms, 1:39, 1:255, 2:451
Photobacterium fischeri, 2:474
Photoisomerization, 2:437
Photosynthesis, **2:436–437,** 2:451
  Chlorophyta, 1:119–120
  chloroplast, 1:120
  photosynthetic microorganisms, 2:437
  Pyrrophyta, 2:470
Photosynthetic microorganisms, **2:437**
  blue-green algae, 1:82–83, 1:119, 1:120, 1:154, 1:203, 1:228, 1:235, 2:436
  Chlorophyta, 1:119–120, 1:348, 2:407, 2:411, 2:460
  gas vacuoles and gas vesicles, 1:235
  Phaeophyta, 1:323–324, 2:421, 2:460
  Pyrrophyta, 2:470
  soil formation, involvement of microorganisms, 2:523
  xanthophylls, 2:605
  Xanthophyta, 2:605–606
  See also Algae
Photosystem I, 2:605
Photosystem II, 2:605
Phycobilins, 2:460, 2:488
Phycobiont, 1:348
Phycocyanin, 1:82
Phycoerythrin, 1:82
Phylogenetic tree, 2:384
Phylogeny, **2:437–438**
Physarum polycephalum, 2:519
Phytophthora infestans, 1:231
Phytoplankton, 2:440
Picornaviruses, 2:580
"Pigging," 2:432
Pili, 1:48, 1:52, 1:133
Pilin, 1:48
Pilobolus, 2:394
"Pink eye," 1:3
Pinocytosis, 1:109
Pipette, **2:438–439,** 2:*439*
Pirosky, Ignacio, 2:391
Pittman, Margaret, **2:440**
Plague, bubonic. *See* Bubonic plague
Plankton and planktonic bacteria, **2:440–441,** 2:616–617
  diatoms, 1:154–155
  photosynthetic microorganisms, 2:437
  red tide, 1:156–157, 2:460, 2:481–482
  See also Zooplankton
Planktonic bacteria, 2:441
Plant alkaloids, chemotherapeutic, 1:117
Plant viruses, 2:441, **2:441–442,** 2:547
Plantar warts, 2:516
Plaque, 1:17, 1:67, 2:387, **2:442**
Plaque assay, 2:434
Plasmids, 1:108, 1:200, 1:230, **2:442–443,** 2:*443,* 2:550
  bacterial artificial chromosome (BAC), 1:48–49
  recombinant DNA molecules, 2:480–481
Plasmodial slime molds, 2:518, 2:519
Plasmodium, 2:363, **2:443–444,** 2:461, 2:463, 2:526
Plasmodium falciparum, 2:363, 2:443, 2:444
Plasmodium malariae, 2:363, 2:443
Plasmodium ovale, 2:363, 2:443
Plasmodium vivax, 2:363, 2:443, 2:444
Platelet-derived growth factor (PDGF), 1:104, 1:106
Pliny the Elder, 2:644
PML. *See* Progressive multifocal leukoencephalopathy
Pneumocystis carinii, 2:445, 2:526
Pneumonia, bacterial and viral, **2:444–445**
  chlamydial pneumonia, 1:118
  defined, 1:17
  Legionnaires' disease, 1:344–346
  Pneumocystis carinii, 2:445, 2:526
  walking pneumonia, 1:118
Podospora anserine, 1:230
Pol I, 2:491
Pol II, 2:491
Polaromonas vacuolata, 1:211
Polio vaccine, 1:186, 2:499, 2:570
Poliomyelitis and polio, **2:445–446**
  Center for Disease Control (CDC), 1:111
  Sabin, Albert, 2:499–501
  Salk, Jonas, 2:501–503
  vaccine, 1:186, 2:499, 2:570
Poliovirus, 2:446
Pollens, allergies, 1:10
Pollution, bioremediation, 1:73–74
Polymerase chain reaction (PCR), 1:240, **2:446–447**
  Actinomyces, 1:3

multiplex PCR, 1:240–241
mycoplasma, 2:408
reverse transcriptase PCR, 1:241
taq enzyme, 2:540–541
Polyomaviruses, 2:584
Pontiac fever, 1:345
Popovic, Mikulas, 1:234
Porin proteins, 2:447
Porins, 2:429, **2:447–448,** 2:*448*
Porphyra, 2:488
Porter, Rodney R., 1:29, 1:175, 1:176, 2:656
Portier, Paul, 1:287
Positional cloning, 1:75
Postherpetic neuralgia, 2:574
Poulik, M.D., 1:176
Pour plate technique, 1:335
Poxviruses, 2:583
Pregnancy
miscarriage, 2:473
reproductive immunology, 2:483–484
Rh incompatibility, 2:487–488
varicella, 2:573
Presumptive tests. *See* Laboratory techniques in microbiology
Prichard, James Cowles, 2:646
Priestley, Joseph, 2:645
Primary wastewater treatment, 2:590
Prion diseases, 2:520
BSE and CJD disease, 1:89–93
Prions, **2:449,** 2:*449,* 2:465, 2:520
Probiotics, **2:450**
anti-adhesion, 1:23–24
Lactobacillus, 1:336–337
See also Microbial flora of the stomach and gastrointestinal tract
Prochloron, 2:436
Progressive multifocal leukoencephalopathy (PML), 2:519, 2:584
Progressive rubella panencephalitis, 2:519
Prokaryotae, **2:450–451**
Prokaryotes, 1:51, 2:450–451
cell cycle and cell division, 1:103–105
cellular respiration, 2:484
chromosomes, 1:122–123
DNA, 1:161
genetic regulation, 1:106–108, 1:244–245
metabolism, 2:377
protein synthesis, 2:454–455
See also Cell cycle (prokaryotic), genetic regulation of; Chromosomes, prokaryotic; Cytoplasm, prokaryotic; Genetic regulation of prokaryotic cells; Prokaryotic membrane transport
Prokaryotic chromosomes, 1:122–123
Prokaryotic membrane transport, **2:451–452**
protein export, 2:453–454
signal hypothesis, 2:515–516
Promoter, 2:548
Prontosil, 2:535
Prophase, 1:103, 1:106–107
Propionibacterium acnes, acne and, 1:2, 1:*2,* 2:380
Propionibacterium granulosum, acne and, 1:2
Prospect Hill virus, 1:259
Protease inhibitors, 1:8
Proteases, HIV and, 1:8
Protein. *See* Protein crystallography; Protein export; Protein synthesis; Proteins and enzymes
Protein crystallography, **2:452,** 2:*453*
Protein electrophoresis. *See* Electrophoresis
Protein export, **2:453–454**
porins, 2:447–448
prokaryotic membrane transport, 2:451–452
signal hypothesis, 2:515–516
Protein synthesis, **2:454–455,** 2:*455*
ribosomes, 2:492
transcription, 1:238, 1:261, 2:486, 2:489, 2:548–549, 2:*549*
translation, 2:551–553
Proteins and enzymes, **2:455–457,** 2:*456*
amino acid chemistry, 1:14–16, 1:*15*
antigenic mimicry, 1:30–31
bacterial membrane and cell wall, 1:52
cell cycle and cell division, 1:103–105
chaperones, 1:113, 1:261, 2:429, 2:582
cytokines, 1:145
dietary, 1:16
electrophoresis, 1:182–183
eukaryotic cell cycle, 1:106–108
prokaryotic cell cycle, 1:108–109
protein crystallography, 2:452
protein export, 2:453–454
protein structure, 1:15
protein synthesis, 2:454–455
structure, 2:*456*
synthesis, 2:454–455, 2:492, 2:551–553
translation, 2:551–553
See also Enzymes
Proteomics, 1:108, 1:244, **2:457–458**
Proteus infection, 1:188
Protista, 1:205, 2:387, 2:450, 2:458, **2:458–462,** 2:*461*
bioluminescence, 1:72–73
chlorophyta, 1:119–120, 1:348, 2:407, 2:411, 2:460
Phaeophyta, 2:421
Pyrrophyta, 2:469–470
Rhodophyta, 2:488
sleeping sickness, 2:517–518
Sporozoa, 2:459, 2:526
Xanthophyta, 2:605–606
Proto-oncogenes, 1:104
Protobacteria, 1:51
Protoplasts and spheroplasts, 1:230, **2:462**
Protozoa, 2:423, 2:459, **2:462–464,** 2:616
cryptosporidium, 1:143–144, 1:315
cysts, 1:119
Entamoeba histolytica, 1:11, 1:12, 1:169, 1:186–187
Giardia, 1:248–249
mastigophora, 2:366–367
paramecium, 2:421
Plasmodium, 2:363, 2:443–444
soil formation, involvement of microorganisms, 2:523
Sporozoa, 2:459, 2:526
Stentor, 2:531, **2:531**
See also Protozoan infection
Protozoan infection, 2:464
blood borne infection, 1:80–82
Chagas disease, 1:111–112
cryptosporidiosis, 1:143–144
giardiasis, 1:248–249
sleeping sickness, 1:178, 2:367, 2:462, 2:517–518
toxoplasmosis, 2:548
See also Protozoa

PrP protein, 1:92, 2:449, 2:465, 2:520
PrPSc protein, 1:90
Prusiner, Stanley, 1:90, 1:92, 2:448, **2:464–465**
Pseudocalanus, 2:616
Pseudomembranous colitis, **2:465**
Pseudomonadaceae, 2:465
Pseudomonas, 1:213, 2:432, **2:465–466**
Pseudomonas aeruginosa, 1:48, 1:68, 1:123, 1:250, 2:386, 2:*412,* 2:465–466
Pseudomonas mallei, 2:465
Pseudomonas stutzeri, 2:411
Pseudoplasmodium, 1:155, 2:518
Psychrophilic bacteria, **2:466,** 2:522
Ptashne, Mark Steven, 2:657
Ptychodiscus brevis, 2:481
Public health, current issues, **2:466–468**
AIDS, 1:7–9, 2:467
anthrax, 1:19–22, 2:467
BSE and CDJ disease, 1:89–93
hemorrhagic fevers, 1:263–264, 2:467
hepatitis and hepatitis viruses, 1:264–267
human immunodeficiency virus, 1:279–280
Lyme disease, 2:468
pertussis, 2:429–430
rabies, 2:477
sexually transmitted diseases (STDs), 2:510–514
tuberculosis, 1:111, 1:123, 1:168, 1:196, 2:467, 2:555–557
wastewater treatment, 2:590
West Nile virus, 2:597–598
World Health Organization (WHO), 2:603–604
Public health. *See* History of public health; Public health, current issues
Puerpueral sepsis, 2:535
Puffballs, 1:232
Pulse-chase experiment, 2:479
Pulsed field gel electrophoresis, 1:122
Purdey, Mark, 1:92
Purkinje, Jan Evangelista, 2:647
Purple non-sulfur bacteria, 2:436
Purple sulfur bacteria, 2:536
Pus, 1:2, 1:3
Puumula virus, 1:259
Pyrex: construction, property, and uses in microbiology, **2:468–469,** 2:*469*
Pyrimethamine, 1:184
Pyrolobus fumarii, 1:211
Pyrrophyta, 1:157, **2:469–470**

Q

Q fever, 1:199, 2:*471,* **2:471–472**
Qualitative and quantitative analysis in microbiology, 1:156, **2:472–474,** 2:*473*
See also Laboratory techniques in microbiology; Microscope and microscopy
Quate, Calvin, 1:36
Queensland tick typhus, 2:493
Quorum sensing, 1:68, **2:474**

R

Rabies, **2:475–477,** 2:*476*
antiserum, 1:32
vaccine, 1:28, 2:569
Rabies vaccine, 1:28, 2:569
Radiation, foods, 1:225, 2:532
Radiation mutagenesis, **2:477–478**
Radiation resistant bacteria, 2:477, **2:478–479,** 2:532
Radioisotopes and their uses, 2:477–478, **2:479–480**
Radiolabeling, 2:479–480
Radiolarians, 2:459
Raji cell assay, 1:286
Raman spectroscopy, 2:525
Ramon, Gaston, 1:289
Rapkine, Louis, 2:397
Rare genotype advantage, **2:480,** 2:506–507
Rat-flea typhus, 2:560
Rat typhus, 2:492
Recombinant DNA molecules, 1:60–62, 1:230, **2:480–481**
Recombination, 1:207, 1:230, **2:481,** 2:657
Red algae, 2:460, 2:462, 2:488
Red blood cells, antigens, 1:27–28
Red-brownish algae, 2:469
Red Queen Hypothesis, 2:480
Red tide, 1:156–157, 2:460, **2:481–482,** 2:*482*
Red water fever, 2:464
Redi, Francisco, 1:246, 2:645
Reduction division, 1:104
Reduviid bugs, 1:111
Reed, Walter, 2:545, 2:651
Refrigeration, 1:66
Reichert, Karl Bogislaus, 2:647
Reindeer lichens, 1:349
Reiter's syndrome, 2:515
Release factors (RF), 2:455
Reoviruses, 2:580
Replication, 1:*163*
enzymes, role in, 2:456–457
virus replication, 2:581–582
Replicative transposition, 2:554
Reproduction, protozoa, 2:463
Reproductive immunology, **2:483–484**
rER. *See* Rough endoplasmic reticulum
Resistance to disease. *See* Infection and resistance
Respiration, **2:484–485**
carbon cycle, 1:100–101
mitochondria and cellular energy, 2:392–393
oxygen cycle, 2:418–419
Respiratory syncytial virus (RSV), 2:585
Restriction enzymes, 1:56, 1:61, 1:182, 1:183, 2:485, 2:*485,* **2:485**
Restriction map, 1:242
Retinoic acid, 1:2
Retroposons and transposable elements, **2:485–486**
Retroviruses, 1:234, **2:486–487,** 2:581, 2:585
antiretroviral drugs, 1:33
human T-cell leukemia virus (HTLV), 1:281
RNA, 2:489
RNA tumor viruses, 2:493–494
as vector in gene therapy, 2:579
Retroviruses, oncogene, 2:415, 2:558
Reverse transcriptase, 2:486–487
Reverse transcriptase PCR, 1:241
Reverse transcription. *See* Transcription
Reye's syndrome, 1:313
RF-1, 2:455
RF-2, 2:455
RF-3, 2:455

Rh and Rh incompatibility, **2:487–488**
Rh disease, 2:487
Rh factor, 1:340, 2:487
Rhabdoviruses, 2:580, 2:585
Rhazes, 2:369
Rhesus disease, 1:28
Rheumatic fever, 2:532
Rheumatoid arthritis, mycoplasma and, 2:408
Rhinitis, 1:10
Rhinovirus, 1:128–129
Rhizobium, 2:383
Rhizobium japonicum, 2:411
Rhizopoda, 2:459
Rhizopus, 2:394, 2:395
Rhizopus nigricans, 1:218
Rhoads, Cornelius, 1:116
Rhodococcus equi, 1:137
Rhodophyta, 2:460, **2:488**
Rhodopseudomonas viridis, 2:436
Rhodopsin, 2:437
Rhodospirillum rubrum, 1:52, 2:436
Ribonucleic acid. *See* RNA
Ribosomal RNA, 2:491–492, 2:551
Ribosomes, 1:146, 2:491, **2:492**
Richet, Charles, 1:287
Richter, Max, 1:339
Rickettsia akari, 2:492
Rickettsia and rickettsial pox, **2:492–493,** 2:560
Rickettsia montana, 2:493
Rickettsia parkeri, 2:493
Rickettsia prowazekii, 2:492, 2:493, 2:560
Rickettsia rickettsii, 2:492, 2:493
Rickettsia tsutsugamushi, 2:492, 2:493, 2:560
Rickettsia typhi, 2:492, 2:493, 2:560
Rift Valley fever, 1:263
Rimantadine, 1:33, 1:116
Rinderpest, 2:648
Ringworm, 2:517
Rivers, THomas, 2:652
RNA, 2:384, 2:486, **2:488–492**
  acridine orange and, 1:2, 1:3
  arenaviruses, 1:34
  bacterial kingdoms, 1:51
  base pairing, 2:*490*
  Berg, Paul, 1:60–62
  Brenner, Sydney, 1:86–87
  Cech, Thomas R., 1:101–102
  Central Dogma, 1:163, 2:*396*
  eukaryotes, 1:204
  life, origin of, 1:349–351
  Miller-Urey experiment, 2:389–390, 2:403
  mitochondrial DNA, 2:393
  origin of life, 1:351
  plant viruses, 2:441
  retroviruses, 2:489
  transcription, 2:548–549
  translation, 2:551–553
  See also Molecular biology and molecular genetics; Mutations and mutagenesis
RNA cancer vaccine, 1:117
RNA polymerases, 1:108, 1:191, 1:244, 2:489, 2:491, 2:548
RNA tumor viruses, **2:493–494**
Robbins, Frederick, 1:185, 1:186, 2:500, 2:596, 2:654
Roberts, Richard John, 2:658
Robertson, O.H., 2:494
Rocky Mountain spotted fever, 1:82, 2:471, 2:492, 2:493
Roentgen, Wilhelm Konrad, 2:650
Roseola, 1:267
Rotavirus, 1:236
Rotavirus gastroenteritis, 1:236
Rotifers, 2:616
Rough endoplasmic reticulum (rER), 2:489
Roundworms, 2:423
Rous, Peyton, 2:493, **2:494–495,** 2:651
Rous sarcoma virus (RSV), 2:493, 2:494, 2:558, 2:585
Roux, Pierre-Paul-Émile, 1:59, 1:272, 1:287, 1:353, **2:495–496**
Roux, Wilhelm, 2:649, 2:650
RSV. *See* Respiratory syncytial virus; Rous sarcoma virus
Rubella virus, 2:519
Rubeola, 2:368
Rüdin, Ernst, 1:256
Ruska, Ernst, 1:179–180, **2:496–497,** 2:651
Ruska, Helmuth, 2:653
Rusts, 1:57
Ryan, Francis, 1:341

S

S layers, 1:53
  See also Sheathed bacteria
Sabia-associated hemorrhagic fever, 1:263
Sabin, Albert, 1:185, **2:499–501**
Sac fungi, 1:232
Saccharomyces carlsbergensis, 2:609
Saccharomyces cerevisiae, 1:230, 2:395, **2:501,** 2:600
Saccharomyces pombe, 2:611
Sagan, Carl, 2:364
Sager, Ruth, 2:655, 2:656
Salk, Jonas, 1:185, 1:340, 2:401, 2:500, **2:501–503,** 2:655
Salmon, Daniel, 1:287
Salmonella, **2:503–504**
  food poisoning, 1:222–225, 2:504–505, 2:558, 2:576
  infections of, 1:188
  invasiveness of, 1:315
  vaccine, 2:504, 2:505
  See also Microbial flora of the stomach and gastrointestinal tract
Salmonella enteritidis, 2:503, 2:505
Salmonella food poisoning, 1:222–225, **2:504–505,** 2:558
Salmonella gallinarum, 2:576
Salmonella typhi, 2:558
Salmonella typhimurium, sensitivity to temperature and pH ranges, 2:404
Salt-loving bacteria, 1:211
Salting, for food preservation, 1:223–224
Salvarsan, 1:218, 2:511
Sambrook, Joseph, 2:657
Sanford, Katherine K., 2:652
Sanger, Frederick, 1:30, 1:55, 2:391, 2:656, 2:658
Santorio, Santorio, 2:644
Sargassum fluitans, 2:421
Sargassum natans, 2:421
Sarin gas, bioterrorism, 1:75
Sawyer, Wilbur A., 2:545
Saxitoxin, 1:157, 2:482
Scanning confocal microscope, 2:473
Scanning electron microscope (SEM), 1:180, 2:388
Scanning tunneling microscope (STM), 2:388

Schatz, A., 1:276
Schaudinn, Fritz, 2:538
Schick, Bela, **2:505–506**
Schistosoma mansoni, 2:597
Schizogony, 2:526
Schleiden, Matthias Jakob, 2:647
Schneider, Franz Anton, 2:648
Schoenheimer, Rudolf, 2:398, 2:607
Scholl, Roland, 1:339
Schonlein, Johann, 2:555
Schott, Otto, 1:1
Schramm, C.H., 2:602
Schramm, Gerhard, 2:655
Schultze, Max Johann, 2:648
Schwann, Theodore, 2:647
Schwartz, Robert, 1:184
Schwerdt, Carlton E., 2:655
SCID. *See* Severe combined immunodeficiency
Scrapie, 1:92, 2:449
Scrub typhus, 1:263, 2:492, 2:493, 2:560
SDS polyacrylamide gel electrophoresis, 1:183
Sea otters, kelp and, 1:323
Sea urchins, kelp and, 1:323
Seaweed
  agar, 1:6, 1:7
  kelp, 1:323–324
Sebaceous glands, acne and, 1:1
Sebum, 1:1
SecB protein, 2:453, 2:454
Secondary immune response. *See* Immunity, active, passive and delayed
Secondary wastewater treatment, 2:590
Sedillot, Charles-Emanuel, 2:649
Selection, **2:506–507**
Selective IgA deficiency, 1:301
SEM. *See* Scanning electron microscope
Semmelweis, Ignaz Philipp, 1:246, 1:283, 2:411, **2:507–508**
Seoul virus, 1:259
Septic shock, 1:44
Septicemic infections, 1:44
Sequestrants, 1:225
Seroconversion, **2:508**
Serological pipette, 2:439
Serology, **2:508–509**
Serratia marcescens, 1:71
Serum sickness, 1:32
Severe combined immunodeficiency (SCID), 1:294, 1:297, **2:509–510**
Sex determination, humans, 1:245
Sex pili, 1:48
Sexually transmitted diseases (STDs), 1:251, **2:510–514,** 2:537–539, 2:589–590
  bacterial, 2:512
  Chlamydia infection, 1:118, 2:512
  Chlamydia trachomatis, 1:118
  genital herpes, 2:511–512, 2:513
  genital warts, 2:513
  gonorrhea, 1:251, 2:510, 2:512
  hepatitis, 2:513
  herpes virus, 2:513
  papillomavirus, 2:513
  syphilis, 1:251, 2:537–538
  See also AIDS
Sharp, Philip Allen, 2:658
Sharpey, William, 1:352
Sheathed bacteria, **2:514,** 2:601
Shellfish, poisoning by, 1:157, 2:482
Shiga-like toxin, 1:171
Shigella, 1:187–188, **2:514–515**
Shigella dysenteriae, 1:168, 2:514
Shigella flexneri, 1:315, 2:514
Shigella sonnei, 2:514
Shigellosis, 1:168
Shingles, 2:574
Shope, Richard, 2:495, 2:653
Short interspersed elements (SINEs), 2:486
Shotgun cloning, 1:49, 1:242, **2:515**
Sick Building Syndrome, 2:408
Side-chain theory, 1:178
Sigma factors, 2:548
Signal hypothesis, 2:453, **2:515–516**
Signal transduction, 1:244
Sigurdsson, Bjorn, 2:519
Simian immunodeficiency virus (SIV), 2:487
Sin Nombre virus, 1:259
SINEs. *See* Short interspersed elements
Singer, S.J., 2:373
Sinsheimer, Robert Louis, **2:516,** 2:656
Sister-chromatids, 1:244
SIV. *See* Simian immunodeficiency virus
Skin. *See* Microbial flora of the skin; Skin infections
Skin infections, **2:516–517,** 2:*517*
  acne, 1:1–2
  See also Microbial flora of the skin
Sleeping sickness, 1:178, 2:367, 2:462, **2:517–518,** 2:*518*
Slime fibrils, 1:249
Slime layer, 1:47
Slime molds, 1:155, 2:461, **2:518–519,** 2:523
Slobber syndrome, 2:395
Slow viruses, **2:519–520**
"Slug," 2:518
Smallpox, **2:520–522,** 2:569
  as bacteriological weapon, 2:521–522
  bioterrorism, 1:76, 2:521–522
  Center for Disease Control (CDC), 1:111
  epidemic, 1:196
  history of, 1:196
  vaccine, 2:568–572
  variola virus, 2:574
Smallpox: eradication, storage, and potential use as a bacteriological weapon, **2:521–522**
Smith, Hamilton, 2:657
Smith, Theobald, 1:287
Smuts, 1:57
Snap freezing, 1:142
Snell, George, 1:288
Snow, John, 1:246
Snow algae, 2:522
Snow blooms, 1:156–157, **2:522**
Sodium hypochlorite, as disinfectant, 1:159
Soil formation, involvement of microorganisms, **2:523**
  Azotobacter, 1:41
  composting, 1:132–133
  lichens, 1:349
  sheathed bacteria, 2:514
Solution-phase hybridization, 1:240
Somatotrophic hormone (STH), 1:104

SOPs. *See* Standard operating procedures
Southern blotting, 2:415
Space science. *See* Extraterrestrial microbiology
Spallanzani, Lazzaro, 2:645
Spanish flu, 1:221
Spectrophotometer, **2:523–524**
Spectroscopy, **2:524–525**
Spencer, Herbert, 2:507
Sphaerotilus natans, 2:514
Spheroplasts, 2:462
Spinae, 1:48
Spinal polio, 2:446
Spirilla, 2:386
Spirochetes, 1:48, 1:52, 2:384, 2:385, **2:525–526**
Spirogyra, 1:119, 2:461
Spirulina, 1:82
Spontaneous abortion, 2:483
Spores, 1:57
Sporocarp, 2:518
Sporogony, 2:526
Sporozoa, 2:459, **2:526**
Sporulation, **2:527**
Spotted fevers, 2:493
Spray drying, 1:223
SRBs. *See* Sulfate reducing bacteria
St. Louis encephalitis virus, 2:597
Stabilizing selection, 2:506
Stahl, Frank W., 2:656
Stahl, Franklin, 2:375
Standard operating procedures (SOPs), 1:260
Stanier, R.Y., 2:458
Stanley, Wendall Meredith, 2:441, **2:527–529,** 2:653, 2:654
Staphylococci and staphylococci infections, **2:529–530,** 2:*530*
  antibiotic resistance, 2:412
  blood agar and hemolytic reactions, 1:80
  toxic shock syndrome, 2:529, 2:547–548
Staphylococcus aureus
  coagulase, 1:125
  enterotoxin, 1:189
  hospital acquired infection, 2:386–387
  toxic shock syndrome, 2:529, 2:534, 2:547–548
Staphylococcus epidermidis
  acne and, 1:2
  coagulase, 1:125
Staphylococcus pyogenes, 2:425
Start codon, 2:491
STDs. *See* Sexually transmitted diseases
Steam heat sterilization, 2:532
Steam pressure sterilizer, **2:530–531**
Stein, William H., 2:657
Steinbach, H. Burr, 1:341
Stentor, **2:531**
Sterilization, 1:31, **2:531–532**
  bacteriocidal, bacteriostatic, 1:54–55
  cold pasteurization, 2:427
  contamination, 1:134
  before culturing, 1:144
  desiccation, 1:154
  disinfection different from, 1:158
  disposal of infectious microorganisms, 1:160–161
  pasteurization, 1:54, 1:246, 1:272, **2:426–427,** 2:532, 2:569
  prions, 1:90
  Pyrex, 2:468–469
  steam pressure sterilizer, 2:530–531
  thermal death, 2:546
Stern, Curt, 2:653
Sternberg, George M., 2:650
STH. *See* Somatotrophic hormone
STI-571, 2:416
Stinkhorns, 1:232
STM. *See* Scanning tunneling microscope
Stomach. *See* Gastroenteritis; Microbial flora of stomach and gastrointestinal tract
Stomach ulcers, 1:11, 1:262, 2:366, 2:381
Stop codon, 2:491, 2:552
Strasburger, Eduard Adolf, 2:649, 2:650
Streak plate technique, 1:335
Strep throat, **2:532–533**
Streptococcal antibody tests, **2:533**
Streptococcal sore throat, 2:532
Streptococci and streptococcal infections, **2:533–535,** 2:*534*
  antibiotics, 2:533, 2:535
  blood agar and hemolytic reactions, 1:80
  division, 1:*50*
  strep throat, 2:532–533
  streptococcal antibody tests, 2:533
Streptococcus mutans, 1:250
Streptococcus pneumoniae, 1:48, 1:195
Streptococcus pyogenes, 1:16, 1:189, 2:425, 2:*534*
Streptococcus thermophilus, 1:337
Streptomyces aureofaciens, 1:116
Streptomycin, 1:116, 2:588
Streptozyme assay, 2:533
Stress proteins, 1:113
Stroma, 1:120
Strominger, Jack L., 2:655
Sturtevant, Alfred Henry, 1:161, 2:652
Sublimation, 1:223
Sugar, for food preservation, 1:223–224
Sulfa drugs, 1:116, 1:220, **2:535**
Sulfadiazine, 2:535
Sulfaguanadine, 2:535
Sulfanilamides, 2:535
Sulfate reducing bacteria (SRBs), 2:432
Sulfathizole, 2:535
Sulfolobus acidocaldarius, 1:211
Sulfonamides, 1:116, 1:220
Sulfur bacteria, 1:115
Sulfur cycle in microorganisms, 1:282–283, **2:536**
Sumner, James B., 1:66, 1:192, 2:654
Superantigen toxin, 1:189, 2:547
Suspended film, 2:590
Sutton, Walter S., 2:651
Swammerdam, Jan, 2:644, 2:645
Sweller, Thomas Huckle, 2:500
Swift, Homer, 1:337
Swine fever, **2:536–537**
Sylvatic yellow fever, 2:613
Symbiosis. *See* Microbial symbiosis
Syme, James, 1:352
Symport, 2:451
Synapomorphies, 2:438
Synchronous growth, **2:537**
Syntrophomonas sp., 1:101

Syphilis, **2:537–538**
epidemic, 1:251
Wasserman test, 1:83, 2:589–590
Szostak, Jack William, 2:658

T

T-8 lymphocytes, 2:593
T-8 suppressor cells, 2:540
T-cell growth factor, 1:234
T-cell leukemia virus. *See* Human T-cell leukemia virus
T cell receptor (TCR), 2:539
T cells (T lymphocytes), 1:288, 1:291, 1:303, **2:539–540**
AIDS, 1:9
allergies, 1:10–11
immune synapse, 1:286–287
immune system, 1:287–288
T delayed hypersensitivity cells, 1:292
T4 phage, 1:55
T phages, 2:577
T suppresser cells, 1:292
Taiwan acute respiratory agent, 1:118
Tamoxifen, 1:116
Tapeworms, 2:423
Taq enzyme, 2:*540,* **2:540–541**
Tatum, Edward Lawrie, 1:175, 1:274, 1:341, 2:382, **2:540–541,** 2:653, 2:654, 2:656
Taxol, 1:116, 2:438
Taxonomy. *See* Microbial taxonomy
Taxus brevifolia, 2:438
Taxus cuspidata, 2:438
TCR. *See* T cell receptor
Teissier, Georges, 2:398
Teliomycetes, 1:57
Telomeres, 1:123
Telophase, 1:103, 1:107, 1:244
TEM. *See* Transmission electron microscope
Temin, Howard, 1:56, 2:657
Temperate phages, 2:433
Temperature sensitivity. *See* Mutants: enhanced tolerance or sensitivity to temperature and pH ranges
Terrorism. *See* Bioterrorism
Tetanolysin, 2:543
Tetanospasmin, 2:543
Tetanus and tetanus immunization, **2:543**
antiserum, 1:32
vaccine, 2:543
Tetanus toxoid, 2:543
Tetracyclines, 1:116, 1:118
Tetrad, 1:105
Thales, 2:643
The Institute for Genomic Research (TIGR), 1:48, **2:544**
Theiler, Max, **2:544–546,** 2:653
Therapeutic cloning, 1:124
Thermal death, **2:546**
Thermal death point, 2:546
Thermophilic bacteria, 1:133, 1:211
Thermophilic fungi, composting, 1:133
Thermotolerant bacteria. *See* Extremophiles
Thermus aquaticus, 1:88, 1:211, 2:540
Thiobacillus ferroxidans, 1:101, 1:115, 2:536
Thiobacillus prosperus, 2:536
Thiobacillus thiooxidans, 1:115, 2:536
Thiosarcina rosea, 2:409
Thiotrix, 1:115, 2:536
Thompson, D'Arcy Wentworth, 2:652
Thrush, 1:261, **2:546–547**
Thylakoid sacs, 1:120
"Thyphoid Mary," 2:559
Thyrotricin, 1:116
Ticks, 1:82, 2:423
Tiger mosquito, 1:153
TIGR. *See* The Institute for Genomic Research
Tijo, Joe Hin, 2:656
Tinea capitis, 2:517
Tinea corporis, 2:517
Tinea cruris, 2:517
Tiselium, Arne, 1:183
Tiselius, 2:651
Tobacco mosaic virus (TMV), 2:441, 2:528, **2:547,** 2:582
Beijerinck, Martinus Willem, 1:59–60, 1:316
Ivanovsky, Dmitri Iosifovich, 1:316
Todd, Alexander, 1:324, 2:489
Togaviruses, 2:580
Tonegawa, Susuma, 2:658
Torovirus, 1:129
Toxic shock syndrome, 2:529, 2:534, **2:547–548**
Toxins. *See* Enterotoxin and exotoxin
Toxoid, 1:32
Toxoplasma gondii, 2:548
Toxoplasmosis, 2:526, **2:548**
Tracking diseases with technology. *See* Epidemiology, tracking diseases with technology
Transcription, 1:238, 1:261, 2:486, 2:489, **2:548–549,** 2:*549*
Transduction, **2:549**
Transfer RNA (tRNA), 2:489, 2:491, 2:551
Transformation, 2:462, **2:549–550**
Transgenics, **2:550–551**
biodegradable substances, 1:66–67
Transient hypogammaglobulinemia, 1:301
Transitions, 2:555
Translation, 1:33, 2:548, **2:551–553,** 2:*552*
Transmembrane proteins, 1:109
Transmission electron microscope (TEM), 1:*179,* 1:179–180, 2:388
Transmission of pathogens, **2:553,** 2:*553*
blood borne infections, 1:80–82
food safety, 1:225–226
hygiene, 1:283–284
Transplantation genetics and immunology, **2:553–554**
cloning, 1:124
history of, 1:307
immunosuppressant drugs, 1:306–307
major histocompatibility complex (MHC), 2:361–363
Transport proteins, 1:109
Transposable elements, 2:485–486
Transposase, 2:485–486
Transposition, 2:485–486, **2:554–555**
Transposons, 1:126, 1:200, 2:554, 2:555
Trematodes, 2:423
Trembley, Abraham, 2:645
Treponema. *See* Syphilis
Treponema pallidum, 1:52, 2:526, 2:589
Treviranus, Gottfried Reinhold, 2:646
Triatomines, 1:111
Trichinella spiralis, 2:423, 2:597
Trichonymphs, 2:462

Trichophyton, 2:517
Trinucleotide repeat mutations, 2:405
Trisomy 21, 1:121
tRNA. *See* Transfer RNA
Trypan red, 1:178
Trypanosoma brucei, 1:81, 2:517
Trypanosoma cruzi, 1:111
Trypanosomes, 1:111, 2:367, 2:462
Trypanosomiasis, 2:517
Tuberculin, 1:327
Tuberculin test, 1:290
Tuberculoid leprosy, 1:346
Tuberculosis, **2:555–557,** 2:*556*
  Center for Disease Control (CDC), 1:111
  chronic disease, 1:123
  Dubos, René, 1:168
  epidemics, 1:196
  history of, 2:555, 2:557
  Koch, Robert, 1:327
  multidrug-resistant tuberculosis (MDR TB), 2:556
  as public health issue, 1:111, 1:123, 1:168, 1:196, 2:467, 2:555–557
  tuberculin test, 1:290
Tularemia, **2:557–558**
Tumor viruses, **2:558**
Turner, J.R., 2:494
Twort, Frederick, 1:55, 2:652
Tyndall, John, 1:273
Type A influenza virus, 1:312
Type B influenza virus, 1:312
Type I interferon, 1:314
Type I restriction enzymes, 2:486
Type II interferon, 1:314
Type II restriction enzymes, 2:486
Type III restriction enzymes, 2:486
Typhoid fever, 1:188, 2:503, **2:558–560**
Typhoidal *Salmonella* infection, 1:188
Typhus, 2:492, 2:493, **2:560–561**
Tyrothricin, 1:167

U

Ulcers, 1:11, 1:262, 2:366, 2:371
Ultra-violet sterilization. *See* Sterilization
Ultrapasteurization, 2:427
Ulva, 2:461
Ulvophyceae, 1:119
Underwood, Michael, 2:446
Undulant fever, 1:206
Urchin barren, 1:323
Ureaplasma urealyticum, 2:408
Urey, Harold, 1:351, 2:389, 2:390, **2:563–565,** 2:*564*
Urinary tract infections, adenoviruses, 1:3
Usnea, 1:349
Ustomycetes, 1:57

V

VacA, 1:252
Vaccination, 1:*289,* **2:567–568,** 2:*568*
  history of, 1:28, 1:319–320
  See also Immunization; Vaccine
Vaccine, **2:568–572,** 2:*571*
  AIDS, 1:9, 2:513, 2:570–571
  arenaviruses, 1:35
  BCG vaccine, 2:432, 2:555–556
  E. coli 0157:H7, 1:23, 1:172
  flu vaccines, 1:313
  foot-and-mouth disease, 1:228
  Haemophilus influenzae, 2:374–375
  hemorrhagic diseases, 1:264
  hepatitis, 1:265, 2:513
  immune stimulation, 1:286
  Junin virus, 1:35
  leprosy, 1:347
  Lyme disease, 1:356
  measles, 2:369
  measles, mumps, and rubella (MMR) vaccine, 2:567
  mumps, 2:403
  pertussis, 2:429–430
  pneumonia, 2:445
  polio, 1:186, 2:499, 2:570
  Q fever, 2:471
  rabies, 1:28, 2:569
  RNA cancer vaccine, 1:117
  rotavirus gastroenteritis, 1:236
  Salmonella, 2:504, 2:505
  sexually transmitted diseases, 2:512–514
  swine fever, 2:537
  tetanus, 2:543
  tularemia, 2:558
  typhoid fever, 2:558–560
  yellow fever, 2:545, 2:614
  See also Immunization; Vaccination
Vaccine gene tun, 1:117
Vacuoles. *See* Gas vacuoles and gas vesicles
Vacuum drying, 1:223
Van Beneden, Edouard, 2:649, 2:650
van Leeuwenhoek, Anton, 1:1, 1:246, 1:273, 1:*343,* **1:343–344,** 2:388, 2:645
van Neil, Cornelius B., 1:325, **2:409,** 2:436, 2:458
Vanterpool, Thomas C., 2:652
Varicella, 2:402–403, **2:572–573**
Varicella zoster virus (VZV), 2:572, **2:573–574,** 2:584
Variola virus, 2:520–521, **2:574**
Variolation, 1:271–272
Varmus, Harold Elliot, 2:658
Vectors
  gene therapy, 2:578–579
  parasitic infection, 2:423
Venereal disease. *See* Sexually transmitted disease
Venezuelan hemorrhagic fever, 1:34, 1:263
Venter, John Craig, 2:544, **2:574–575,** 2:659, 2:660
Verotoxin, 1:171
Vesalius, Andreas, 2:644
Vesicles. *See* Gas vacuoles and gas vesicles
Veterinary microbiology, **2:575–577,** 2:*576*
  foot-and-mouth disease, 1:227–228, 1:354
  rabies, 2:475–477
  swine fever, 2:536–537
  tracking diseases with technology, 1:199–200
  See also Animal models of infection; Zoonoses
Viable but nonculturable bacteria, **2:577**
Vibrational spectroscopy, 2:525
Vibrio cholerae
  cholera, 1:193

enterotoxin, 1:189
sensitivity to temperature and pH ranges, 2:404
Vibrio fischeri, 1:354
Vibrio furnisii, 1:118
Vibrio parahaemolyticus, 1:47
Vinblastine, 1:117
Vincristine, 1:117
Vinograd, Jerome, 2:375
Viral classification, 2:579
Viral epidemics. *See* Epidemics and pandemics; Epidemics, viral
Viral gastroenteritis, 1:236
Viral genetics, 2:438, **2:577–578**
Asilomar conference, 1:36
bacteriophages and bacteriophage typing, 1:55–56
latent viruses and disease, 1:340–341
lysogeny, 1:356–357
oncogene, 2:415
phage genetics, 2:433–434
phage therapy, 2:434
phylogeny, 2:437–438
plant viruses, 2:441–442
radiation mutagenesis, 2:477–478
retroviruses, 2:486–487
RNA tumor viruses, 2:493–494
slow viruses, 2:519–520
transduction, 2:439
See also Microbial genetics; Viral vectors in gene therapy
Viral infection
AIDS, 1:7–9
antiviral drugs, 1:33
arenavirus, 1:34–35
blood borne infections, 1:80–82
cats, 2:575
Centers for Disease Control (CDC), 1:110–112
chickenpox, 2:572–573
common cold, 1:127–128
dogs, 2:575
enterovirusinfection, 1:189–190
environmental contamination, 1:136–137
epidemics, 1:193–194, 1:196–198
eye infections, 1:212–213
gastroenteritis, 1:236
hand-foot-mouth disease, 1:258
hantavirus and Hanta disease, 1:258–259
hemorrhagic fevers and diseases, 1:263–264
hepatitis, 1:264–267
human immunodeficiency virus, 1:279–280
immune system, 1:287–288
infection and resistance, 1:308–310
infection control, 1:310–311
invasiveness and intracellular infection, 1:315
latent viruses, 1:340–341
Lichen planus, 1:348
measles, 2:368–369
meningitis, 2:374–375
mononucleosis, 2:399
mumps, 2:402–403
pneumonia, 2:444–445
poliomyelitis and polio, 2:445–446
rabies, 2:475–477
retroviruses, 2:486–487
rheumatic fever, 2:532
sexually transmitted diseases (STDs), 2:510–514
skin infections, 2:516–517
slow viral infection, 2:519
smallpox, 1:76, 1:196, 2:520–522, 2:568–572
strep throat, 2:532
swine fever, 2:536–537
T cells (T lymphocytes), 2:539–540
transduction, 2:439
varicella, 2:572–573
varicella zoster virus, 2:573–574
variola virus, 2:520–521, 2:574
West Nile virus, 2:597–598
yellow fever, 2:613–614
See also Plant viruses
Viral infections
chemotherapy, 1:116–117
cowpox, 1:138
transmission of pathogens, 2:553
Viral pneumonia, 2:444–445
Viral vectors in gene therapy, **2:578–579**
phage therapy, 2:434
retroviruses, 2:486–487
Virchow, Rudolf, 1:247, 2:648
Virology, viral classification, types of viruses, **2:579–581**
Centers for Disease Control (CDC), 1:110–112
epidemics, 1:196–198
latent viruses and disease, 1:340–341
oncogene, 2:415
plant viruses, 2:441–442
See also Viral genetics; Viral infection; Viral vectors in gene therapy; Virus replication; Viruses and responses to viral infection
Virus replication, **2:581–582,** 2:*582*
bacteriophages and bacteriophage typing, 1:55–56
Beijerinck, Martinus Willem, 1:59–60
herpes and herpes virus, 1:267–268
interferons, 1:313–314
lysogeny, 1:356–357
oncogene, 2:415
retroviruses, 2:486–487
Viruses, **2:582–585**
adenoviruses, 1:3–4, 2:581, 2:584
AIDS, 1:7–9
Andes virus, 1:259
antiviral drugs, 1:33
arenavirus, 1:34–35
Arenaviruses, 1:34–35, 1:263
Asfivirus, 2:536
Bayou virus, 1:259
Birnaviruses, 2:580
Black Creek Canal virus, 1:259
Blue River virus, 1:259
Bunyavirus group, 1:263
cauliflower mosaic virus (CMV), 2:515
Centers for Disease Control (CDC), 1:110–112
classification of, 2:577, 2:579
cold viruses, 1:128–129
common cold, 1:127–128
contamination, 1:135–136
Coronavirus, 1:129, 2:575
Coxsackie virus group, 1:258
Ebola virus, 1:81, 1:*81,* 1:172–173, 1:*173,* 1:264, 2:585, 2:657
enterovirus infections, 1:189–190
epadnaviruses, 1:264

epidemics, 1:193–194, 1:196–198
Epstein-Barr virus, 1:82, 1:201, 1:267, 2:399, 2:558
evolution of, 1:209
evolutionary origin, 1:208–209
feline leukemia virus (FELV), 2:487, 2:575
Filoviruses, 1:172–173, 1:263
Flaviviruses, 1:263, 2:585
hantavirus, 1:258–259
hemorrhagic fevers, 1:263–264
hepadnaviruses, 1:264, 2:584
hepatitis viruses, 1:264–267, 2:558, 2:580, 2:584
herpes virus, 1:267–268, 2:513, 2:581
human immunodeficiency virus, 1:279–280
human papillomavirus (HPV), 2:516, 2:558, 2:584
human T-cell leukemia virus (HTLV), 1:233, 1:281, 2:493, 2:519, 2:558, 2:585
immune system, 1:287–288
infection cycle of, 2:583
influenza virus, 1:262, 1:312, 1:*312,* 2:570
JC papovirus, 2:519
latent viruses, 1:340–341
latent viruses and disease, 1:340–341
lymphoadenopathy-associated virus (LAV), 2:400
maedi-visna virus, 2:519
Marburg virus, 1:264, 2:585
measles virus, 2:368, 2:519
mumps virus, 2:402
Myxoma virus, 2:507
orthomyxoviruses, 1:311–312, 2:580, 2:585
papovaviruses, 2:581, 2:584
parainfluenzae virus, 2:575
paramyxovirus group, 2:368
parvoviruses, 2:580, 2:584
Pestivirus, 2:536
Picornaviruses, 2:580
plant viruses, 2:441–442
poliovirus, 2:446
polyomaviruses, 2:584
poxviruses, 2:583
Prospect Hill virus, 1:259
Puumula virus, 1:259
reoviruses, 2:580
respiratory syncytial virus (RSV), 2:585
retroposons, 2:486
retroviruses, 1:234, 2:486–487, 2:581, 2:585
rhabdoviruses, 2:580, 2:585
Rhinovirus, 1:128–129
RNA tumor viruses, 2:493–494
Rous sarcoma virus (RSV), 2:493, 2:494, 2:558, 2:585
rubella virus, 2:519
Seoul virus, 1:259
Sin Nombre virus, 1:259
slow viruses, 2:519–520
St. Louis encephalitis virus, 2:597
tobacco mosaic virus (TMV), 1:59–60, 1:316, 2:441, 2:528, 2:547, 2:582
Togaviruses, 2:580
Torovirus, 1:129
transmission of pathogens, 2:553
tumor viruses, 2:558
varicella, 2:572–573
varicella zoster virus, 2:573–574
variola virus, 2:520–521, 2:574
West Nile virus, 2:597–598
yellow fever virus, 1:58, 2:613
See also Plant viruses; Viral genetics; Viral infection; Viral vectors in gene therapy; Virology, viral classification, types of viruses; Virus replication
Viruses and responses to viral infection, **2:582–585**
Vitritis, 1:212
VM-16, 1:117
Volvox, 2:460
von Baer, Karl Ernst, 2:646
von Bamberger, Eugen, 1:339
von Behring, Emil, **1:58–59,** 1:178, 1:272, 1:287, 1:325
von Euler-Chelpin, Hans, **1:205–206**
von Frerichs, Friedrich, 1:177
von Gruber, Max, **1:255–256,** 1:339
von Haller, Albrecht, 2:645
von Kölliker, Albrecht, 2:650
von Liebig, Justus, 2:647
von Mohl, Hugo, 2:647
von Möllendorff, Wilhelm, 1:330
von Nageli, Carl Wilhelm, 1:255
von Pettenkoffer, Max Josef, 1:252, 1:255
von Pirquet, Clemens, 2:651
von Plenciz, Marcus Anton, Sr., 2:645
von Siebold, Karl Theodor Ernst, 2:647
von Tschermak, Erich, 2:651
von Wasserman, August Paul, 1:83, 2:511, 2:538, **2:589**
Vozrozhdeniye Island, **2:585**
VP-16, 1:117
VZV. *See* Varicella zoster virus

W

W. bancrofti, 2:423
Waksman, Selman Abraham, 1:116, 1:167, 1:276, **2:587–589,** 2:*588,* 2:653
Waldeyer, Heinrich Wilhelm, 2:650
Walking pneumonia, 1:118
Wallace, Alfred Russell, 2:647
Wannamaker, Lewis, 1:338
Warburg, Otto, 1:330
Warner, Noel, 2:656
Warren, Robin, 2:366
Warts, 2:516
Wasserman, August Paul von, 1:83, 2:511, 2:538, **2:589**
Wasserman test, 1:83, **2:589–590**
Wastewater treatment, **2:590,** 2:*591*
dysentery, 1:168–170
fermentation, 1:218
See also Water pollution and purification; Water quality
Water molds, 1:232, 2:461
Water pollution and purification, **2:591–592,** 2:*592*
carbon cycle, 2:419
cryptosporidium and cryptosporidiosis, 1:143
dysentery, 1:168–170
Stentor, 2:531
wastewater treatment, 2:590–591
zooplankton, 2:616–617
See also Water quality
Water quality, **2:592–594,** 2:*593*
Campylobacter jejuni, 1:99–100
chlorination, 1:118–119, 1:119–120
contamination, 1:136–137

cryptosporidium and cryptosporidiosis, 1:143
dysentery, 1:168–170
Giardia and giardiasis, 1:248–249, 2:367
indicator species, 1:308
laboratory techniques, 1:335
Stentor, 2:531
wastewater treatment, 2:590–591
See also Water pollution and purification
Waterfleas, 2:616
Watson, James D., 1:114, 1:138, 1:*139,* 1:162, 1:163, 1:269, **2:594–595,** 2:*595,* 2:655, 2:659
Weigert, Carl, 1:253
Weismann, August F., 2:649, 2:650
Weissmann, Charles, 2:465
Welch, William Henry, **2:595–596**
Weller, Thomas, 1:185, 1:186, 2:424, **2:596–597,** 2:654
Wells, William Charles, 2:646
West Nile virus, **2:597–598**
Western blots, 1:183
"White smokers," 1:282
Whiteheads, 1:2
Whittaker, Robert, 2:458
WHO. *See* World Health Organization
Whole genome shotgun cloning, 2:515
Whooping cough. *See* Pertussis
Widal, Georges Fernand Isidor, 1:255
Wiener, Alexander, 1:340
Wilcox, Kent, 2:657
Wilkins, Maurice Hugh Frederick, 1:162, **2:598–599,** 2:656
Willadsen, Steen A., 2:658
William of Saliceto, 2:644
Williams, Robley C., 2:655
Wilmut, Ian, 2:660
Wine making, 1:217–218, 2:424–425, **2:599–601**
Winogradsky column, **2:601**
Winogradsky, Sergei, 1:167
Wittgenstein, Annelise, 1:330
Woese, Carl R., 2:450, 2:658
Wöhler, Friedrich, 1:66, 2:646
Wolff, Kaspar Friedrich, 2:645
Wollman, Élie, 1:317–318, 2:656
Wong-Staal, Flossie, 2:*601,* **2:601–602**
Woodward, Robert B., **2:602–603**
World Health Organization (WHO), 1:275, 2:522, **2:603–604,** 2:654, 2:656
Wright, Almroth Edward, 1:218, 2:435, **2:604**

X

X-linked agammaglobulinemia, 1:293, 1:301
X-ray fluorescence, 2:525
X-ray photoelectron spectroscopy, 2:525
Xanthophylls, 2:488, **2:605**
Xanthophyta, **2:605–606**
Xanthoria, 1:349
Xenorhabdus luminescens, 1:354
Xenorhabdus nematophilus, 2:383

Y

YAC. *See* Yeast artificial chromosome
Yalow, Rosalyn Sussman, **2:607–609**
Yanofsky, Charles, 2:657
Yeast, 1:231, 2:*609,* **2:609–610**
aerobes, 1:5
asexual generation and reproduction, 1:35
cell cycle of, 2:611
colony and colony formation, 1:129–130
cryoprotection, 1:141–142
cryptococci and cryptococcosis, 1:142–143
fermentation, 1:217–218
hyphae, 1:284
life cycle of, 2:609
protoplasts and spheroplasts, 2:462
types of, 2:599
wine making, 2:599
See also Yeast, economic uses and benefits; Yeast, infectious; Yeast artificial chromosome; Yeast genetics
Yeast, economic uses and benefits, 1:217–218, **2:611–612**
Yeast, infectious, 2:*612,* **2:612–613**
eye infections, 1:213
Lichen planus, 1:348
skin infections, 2:516–517
thrush, 2:547
transmission of pathogens, 2:553
See also Mold
Yeast artificial chromosome (YAC), 2:515, **2:610–611,** 2:659
Yeast genetics
Saccharomyces cerevisiae, 2:501
shotgun cloning, 2:515
yeast artificial chromosome (YAC), 2:610–611
See also Mold
Yellow fever, 1:263, 2:423, **2:613–614**
history of, 2:545
infection control, 1:310
treatment of, 2:614
vaccine, 2:545, 2:614
Yellow fever virus, 1:57–58, 2:613
Yellow-green algae, 2:605–606
Yersin, Alexander, 1:93, 1:272, 1:287, 1:353, 2:496, 2:650
Yersinia, 1:188
Yersinia enterocolotica, 1:188
Yersinia pestis, 1:93, 1:188, 1:195, 2:644
Yersinia pseudotuberculosis, 1:188
Yogurt, 1:337
Young, John, 2:370
Yuan, Robert, 2:375

Z

Zaug, Arthur, 1:102
Zeiss, Carl, 1:1
Zeiss Works, 1:1
Zidovudine, 1:8
Ziehl-Neelsen stain, 1:335
Zimmer, Esther, 1:*342,* 1:343
Zinc fingers, 1:9
Zinder, Norton, 1:55, 1:342, 2:654, 2:655
Zinsser, Hans, 1:337
ZoBell, Claude Ephraim, 2:431, **2:615**
Zoo FISH, 1:222
Zoomastigina, 2:459
Zoonoses, **2:615–616,** 2:*616*
arenaviruses, 1:35
bubonic plague, 1:93, 1:*94,* 1:95, 1:188, 1:193–194, 1:195, 1:274
campylobacteriosis, 1:99–100

Chagas disease, 1:111–112
cowpox, 1:138
dengue fever, 1:153–154
Ebola virus, 1:172–173
Plasmodium, 2:363, 2:443–444
Q fever, 2:471–472
toxoplasmosis, 2:548
transmission of pathogens, 2:553
tularemia, 2:557–558
typhus, 2:560–561
veterinary microbiology, 2:575–577
West Nile virus, 2:597–598
yellow fever, 2:613–614
See also Animal models of infection; Veterinary microbiology
Zooplankton, 2:440, **2:616–617**
See also Plankton and planktonic bacteria
Zooxanthellae, 2:470
Zovirax, 1:184
Zworykin, Vladimir Kosma, 1:180
Zygomycetes, 1:284, 2:407
Zygosporangium-forming fungi, 1:232
Zygotes, 1:105, 1:121
Zyloprim, 1:184